9th Farnsworth Lecture , March 25th 2022

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NIH Special Volunteer

Are microbes the "true sources" of bioactive compounds isolated from multi-celled organisms such as plants and marine invertebrates?

Nominal Organization of the Lecture

Due to the main emphasis of the Farnsworth lectures being directed towards the "Plant Kingdom" I will discuss that area in some depth.

Then I will follow with some examples (old and new), of bioactive compounds isolated from marine invertebrates, that are definitively produced by commensal microbes though frequently these are "as yet uncultivated".

Growth Conditions / Compounds

From the very earliest days of TCM, the conditions of plant growth can and do alter the levels/types of biological activity (read secondary metabolites found on isolation), thus one SHOULD/MUST take these climatic and geographic differences into account when comparing isolated secondary metabolites whether from plants, endophytes/endophytes or the "whole gemisch".

However, how many times is this done before publication ?

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) 30+ years, there have been a number of reports in the literature that have invoked endophytic microbes 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.

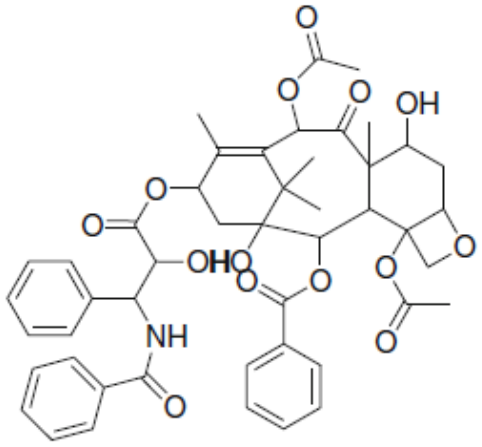
This was followed over the years by reports on the vincas, podophyllotoxin and camptothecin.

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

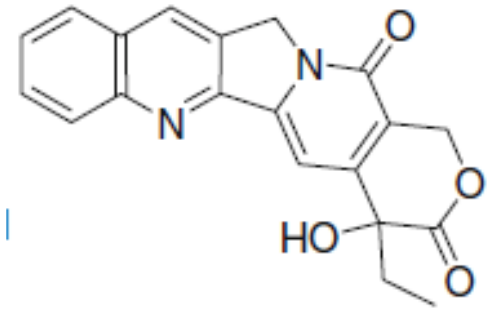
Plant – Microbe Axis II

In Phytochemical Reviews in 2012, Kaul et al, listed > 90 bioactive compounds that had been isolated from endophytes of medicinal plants.

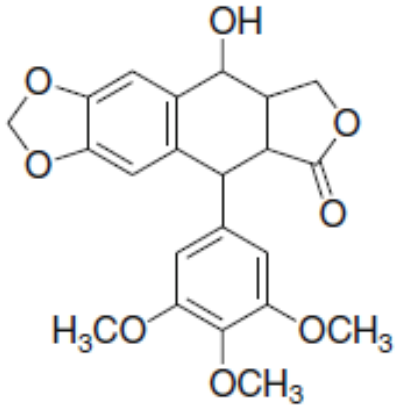
I have deliberately only shown the three well known ones as a "sampler" of what is / are present.



Taxol

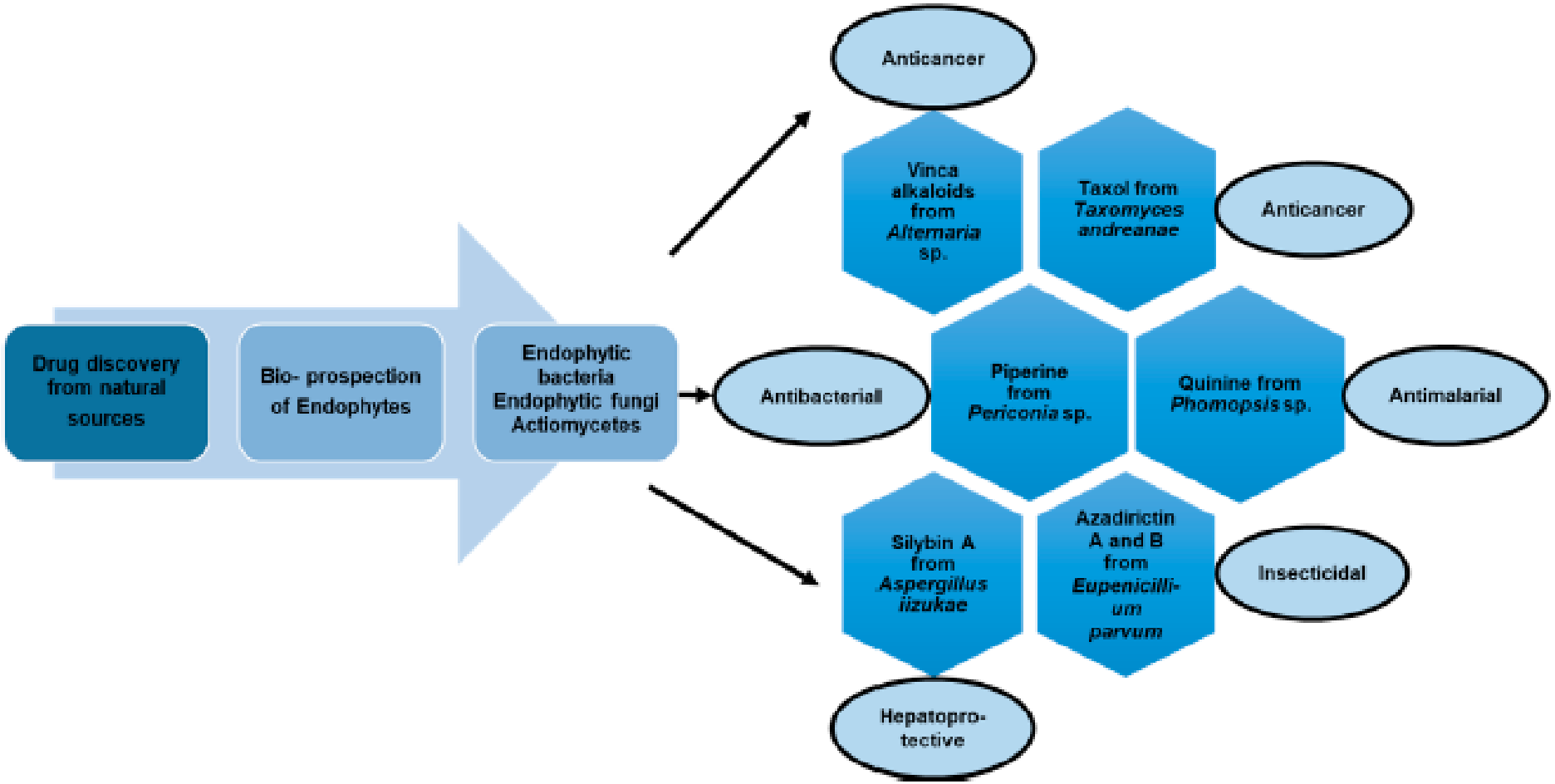


Camptothecin plus precursors



Podophyllotoxin

Idealized Flow Diagram for Investigating Endophytes



Tiwari and Bae, Microorganisms, 2022, 10, 360

Sources of Error

TABLE 1 | Possible sources of error when working with endophytic fungi and commonly used or suggested preventive measures.

No	Experimental step	Risk of errors	Preventive measures
1	Isolation of fungus	• Sampling from diseased plants • Improper surface sterilization • Contamination with fungi from laboratory	• Properly document sampling site and deposit voucher specimen. • Include the solution of the "last wash" in all following experimental steps (PCR, cultivation on liquid and solid media). • Use appropriate aseptic techniques and regular controls for spore contamination of workspaces.
2	Secondary metabolite measurement from cultivated endophytic fungi	False positive detection caused by the carry-over of plant metabolites or enzymes	• Perform time course experiment to observe the secondary metabolite titer increase with biomass formation; possibly observed over several passages. • Include a negative control in the fermentation with added fungicides to detect any carry-over of plant secondary metabolites or enzymes that may contribute to the formation of the secondary metabolite. • Modulate secondary metabolite production under different culture conditions (should only be considered as hard evidence when combined with other controls, see above).
3	PCR amplification of putative biosynthetic genes	Contamination of extracted DNA with plant DNA, or the DNA of other microorganisms	• Include water controls during DNA extraction and PCR to control for reagent contamination • Include PCR reactions targeting plant housekeeping genes or taxonomic markers to check for plant DNA contamination • Use quantitative PCR against the gene of interest and a fungal housekeeping gene to compare copy numbers. • Generate vector-based genome libraries of the fungus to eliminate DNA fragments of low abundance

Sagita et al, Front, Bioeng. Biotech, 2021, 9, 64906

Environmental Factors with Plants/Endophytic Bacteria

TABLE 1 | Factors affecting the community structure of endophytic bacteria in medicinal plants.

Habitat	Representative host plant	Isolated part(s)	Factor(s)	Factor(s) explanatory comments	References
Mountains in subtropics	*Caragana jubata*	Root	Environment: altitude	Different dominant endophytic bacteria	Xu et al., 2014
Mountains in subtropics	*Stellera chamaejasme*	Leaf, stem, and root	Tissue	The OTUs number of endophytic bacteria from high to low in different tissues were leaf > stem > root	Jin et al., 2014
Karst landform	*Cyrtomium fortunei*	Root	Environment: soil type	The highest endophyte numbers were observed in low calcium soil	Li F. et al., 2019
Grassland habitat in savanna	*Baccharis dracunculifolia*	Root and leaf	Tissue	The OTUs number of endophytic bacteria from high to low in different tissues were root > leaf	Santana et al., 2016
Plantation	*Paullinia cupana*	Leaf	Health status of plants	Lower relative abundance in healthy plants than in susceptible plants	Bogas et al., 2015
Temperate maritime climate islands	*Pseudowintera colorata*	Leaf, stem and root	Tissue age	The species richness of endophytic bacteria increased with tissue age	Purushotham et al., 2020
Temperate forest	*Cinnamomum camphora*	Leaf	Season	The order of the endophytes richness in the samples was spring > summer > early winter	Elmagzob et al., 2019
Subtropical region	*Morus* sp.	Branch	Season	Spring samples harbor higher bacterial OTUs, α-diversity, and bacterial community complexity than autumn samples	Ou et al., 2019
Mediterranean region	*Helianthus annuus*	Root	Environment: moisture	Endophyte colonization was positively correlated with humidity	Santos et al., 2014
Subtropical botanical gardens	*Sarracenia* spp.	Rhizome	Taxonomy of plants	Different dominant endophytic bacteria	Sexton et al., 2019

Wu et al, Front. Plant. Sci., 2021, 12, 646146

Important Drugs from Endophytic Fungi

Table 6. Endophytic fungi that produce important therapeutic drugs.

Secondary Metabolite	Representative Endophytic Fungi	References
Paclitaxel (anticancer chemotherapy drug)	*Aspergillus candidus*, *Chaetomella raphigera*, *Cladosporium cladosporioides*, *Cladosporium oxysporum*, *Lasiodiplodia theobromae*, *Penicillium aurantiogriseum*, *Periconia* sp., *Pestalotiopsis microspora*, *Pestalotiopsis versicolor*, *Phoma betae*, *Phomopsis* sp., *Phomopsis* sp., *Phomopsis* sp., *Phyllosticta citricarpa*, *Phyllosticta melochiae*	[168–170,198–207]
Camptothecin and analogs (anticancer chemotherapy drug)	*Fusarium solani*, *Fusarium oxysporum*, *Entrophospora infrequens*, *Trichoderma atroviride*, *Neurospora* sp., *Alternaria alstroemeriae*, *Alternaria burnsii*, *Alternaria* sp., *Alternaria alternata*, *Xylaria* sp., *Aspergillus* sp., *Aspergillus niger*	[187–190,208–215]
Vinblastine and vincristine (anticancer chemotherapy drug)	*Alternaria alternata* sp, *Fusarium oxysporum*, *Talaromyces radicus*, *Curvularia verruculosa*, *Botryosphaeria laricina*	[177–179,216,217]
Podophyllotoxin (anticancer chemotherapy)	*Phialocephala fortinii (0.5 to 189 µg/L)*, *Alternaria tenuissima*, *Mucor fragilis*, *Trametes hirsuta*, *Alternaria* sp. *Fusarium solani*	[218–223]
Fusidic acid (antibiotic)	*Acremonium pilosum*	[195]

Galindo-Solis and Fernandez, Microorg., 2022, 10, 339

Compounds Isolated from Endophytic Fungi 2011-2021

No.	Endophytic Fungus	Host Plant	Regions/Countries	Compound	Biological Activity	Ref.
1	*Lophiostoma* sp.	*Eucalyptus exserta*	Guangzhou, China.	Scorpinone	Antibacterial	[22]
2	*Mycosphaerella* sp.	*Myrciaria floribunda*	Amazon rainforest, Brazil.	Myriocin	Antifungal	[23]
3	*Mucor* sp.	*Centaurea stoebe*	Idaho, USA	Terezine E	Antifungal and cytotoxicity	[24]
4	*Aspergillus calidoustus*	*Acanthospermum australe*	Jalapao State Park, Tocantins, Brazil.	Ophiobolin K 6-epi-ophiobolin K	Antifungal, trypanocidal and cytotoxicity	[25]
5	*Phomopsis* sp.	*Garcinia kola (Heckel) nut*	Yaounde, Cameroon	Cytochalasins H	Antibacterial and cytotoxicity	[26]
6	*Aspergillus nidulans*	*Nyctanthes arbor-tristis Linn*	Karachi, Pakistan	Sterigmatocystin	Antiproliferative activity	[27]
7	*Trichoderma asperellum* and *Trichoderma brevicompactum*	*Vinca herbacea*	Hamedan, Iran	4b-hydroxy-12,13-epoxytrichothec-9-ene	Antimicrobial and antiproliferative activity	[28]
8	*Phyllosticta elongata*	*Cipadessa baccifera*	Western Ghats, India	Camptothecin	Anticancer agent	[29]
9	*Fusarium verticillioides*	*Huperzia serrata*	Gucheng Mountain, Sichuan, China	Huperzine A	Treatment of Alzheimer's disease	[30]
10	*Fusarium solani*	*Cassia alata*	Bangladesh	Napthaquinones Azaanthraquinones	Cytotoxicity, antimicrobial and antioxidant activity	[31]
11	*Fusarium* sp. and *Lasiodiplodia theobromae*	*Avicennia lanata*	Terengganu, Malaysia	Anhydrofusarubin dihydrojavanicin	Antitrypanosomal	[32]
12	*Corynespora cassiicola*	*Gongronema latifolium*	Nigeria	Corynesidone D	Anti-inflammatory/ anticancer agent	[33]
13	*Pestalotiopsis theae*	*Camellia sinensis Theaceae*	Hangzhou, China	punctaporonin H	Antibacterial and cytotoxicity	[34]
14	*Phialocephala fortinii*	*Podophyllum peltatum*	Tamilnadu, India	Podophyllotoxin	Antiviral, antioxidant, and antirheumatic activities	[35]

This article reports 220 new compounds with rare or novel structures or skeleton structures from endophytic fungi from 82 journal articles between 2011 and 2021 and briefly describes the sources of endophytic fungi, chemical structures, and biological activities of these compounds.

Wen et al, J. Fungi, 2022, 8, 205

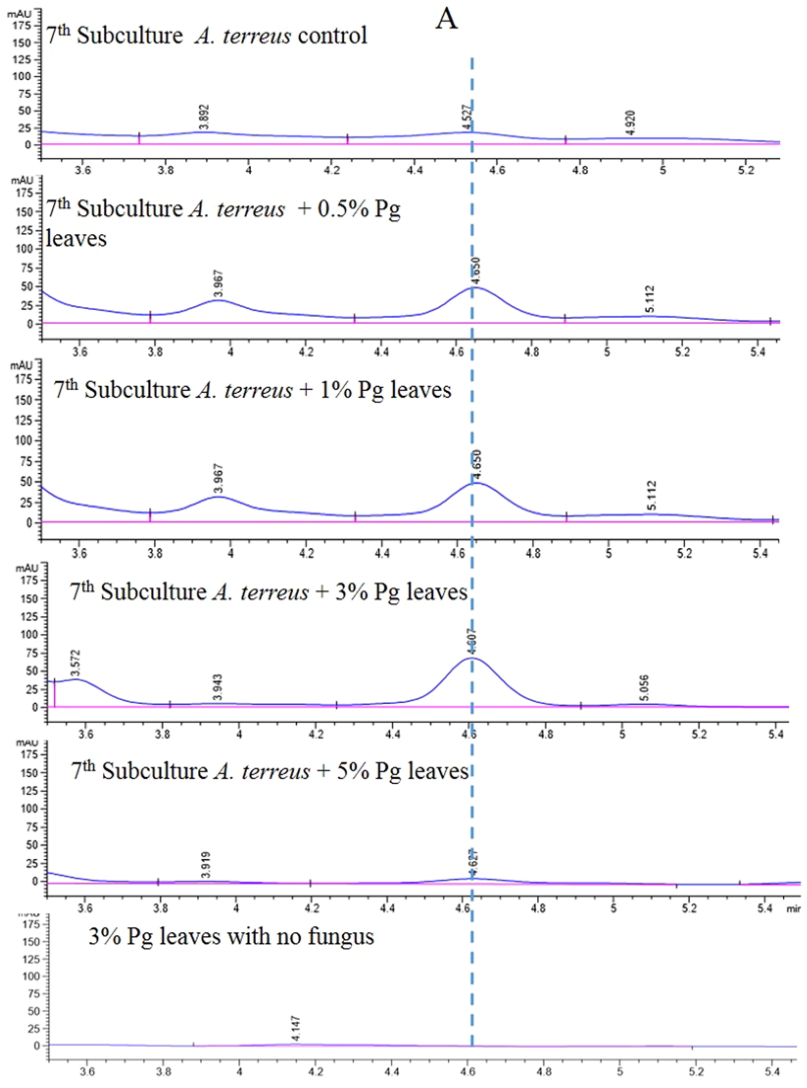
Yields of Compounds Isolated from Endophytic Fungi 2011–2021

No.	Endophytic Fungus	Host Plant	Culture Conditions	Secondary Metabolites	Yield	Ref.
1	*Hansfordia biophila*	*Hedychium acuminatum* Roscoe	Inoculated in potato glucose broth (PDB) medium and shaken at 120 rpm at 25 °C for 7 days.	Tannin	41.6 μm·mL^{-1}	[121]
2	*Aspergillus terreus*	*Ficus elastica*	Inoculated into PDB medium and incubated at 30 °C for 20 days on a rotatory shaker incubator at 140 rpm.	Camptothecin	320 μg/L	[122]
3	*Guignardia mangiferae* HAA11	*Taxus x media*	Inoculated into (PDB) medium and incubated at 200 rpm at 28 °C for 5 days.	Paclitaxel	720 ng/L	[123]
4	*Papulaspora* sp.S6	*Phellodendron amurense* Rupr	Mutagenesis by UV, X-ray rays, and $NaNO_2$, inoculated in PDB medium, and shaken at 100 rpm at 28 °C for 7 days.	Berberine	12.28 mg/L	[124]
5	*Actinoplanes teichomyceticus*		Improvement of the output of teicoplanin by genome shuffling; Inoculated teicoplanin medium and cultured at 28 °C for 15–20 days.	Teicoplanin	3016 μm·mL^{-1}	[125]
6	*Phialocephala fortinii*	*Podophyllum peltatum*	Inoculated in Sabouraud's dextrose agar (SDA) and cultured at 23 °C for 4–6 weeks.	Podophyllotoxin	189 μg/L	[126]
7	*Entrophospora infrequens* RJMEF001	*Nothapodytes foetida*	Inoculated into wheat bran containing Sabouraud's broth, and incubation was carried out at 28 ± 2 °C for 28 days.	Camptothecin	503 ± 25 μg/100 g dry cell mass (in Sabouraud broth)	[127]
8	*Epicoccum nigrum* SZMC 23769	*Hypericum perforatum*	Fungal isolates were grown in potato dextrose broth (PDB) for 7 days at 25 °C.	Hypericin, Emodin	117.1 μg/mL, 87.7 μg/mL	[128]

Wen et al, J. Fungi, 2022, 8, 205

Taxol post Stierle / Strobel

Complementation of Taxol Fermentations



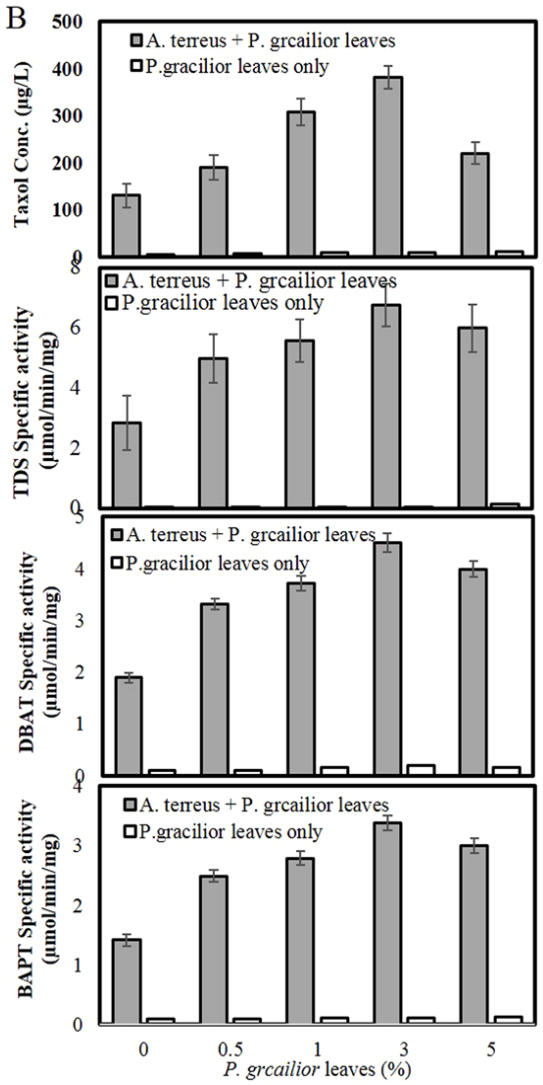
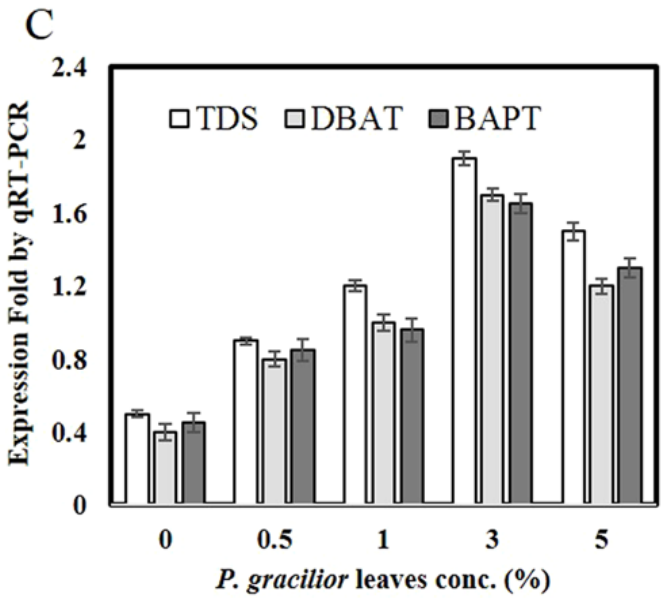


Figure 3 Surface sterilized P. gracilior leaves restored the Taxol productivity of A. terreus. 7th fungal subculture was grown on MID medium, incubated for 10 days, then amended with surface sterilized leaves of P. gracilior at 0.5, 1, 3 and 5% (w/v), incubated for 20 days.

Taxol was extracted and quantified by HPLC (A).

The activities of Taxol biosynthesis rate-limiting enzymes, TDS, DBAT and BAPT (C) were assessed.



El-Sayed et al, Sci. Rept. 2019, 9, 11534

Fungal Endophytes that have Taxol Production Potential

Table 6 Dry biomass (g L^{-1}) and taxol production (µg L^{-1} culture filtrate) by immobilized mycelia of *E. nigrum* TXB502–GR33 grown for six different fermentation cycles

Number of cycles	Dry biomass(g L^{-1})	Taxol conc. (µg L^{-1})
1	–	1372.49 ± 18.45[a]
2	–	1371.64 ± 12.47[a]
3	–	1368.81 ± 21.39[a]
4	–	1361.29 ± 15.51[b]
5	–	1355.33 ± 20.29[b]
6	3.51 ± 0.16	1348.21 ± 23.61[c]
Total	3.51 ± 0.16	8187.77 µg/6 L

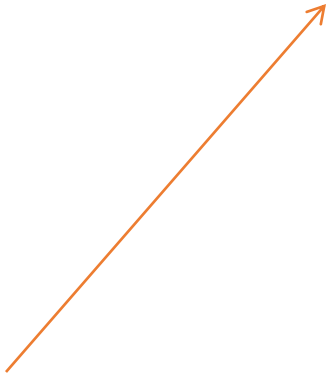
Cultures were grown in 50 mL of the modified FBM medium (pH 6.0) inoculated with 50 beads and incubated at 120 rpm and 25 °C for 14 days. Calculated mean is for triplicate measurements from two independent experiments. [a–c] means with different superscripts in the same column are considered statistically different (LSD test, $P \leq 0.05$)

El-Sayed_et al App Microbiol, Biotech, 2020, 104, 6991-7003

Some Relevant Comments on Fungal Taxol I

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.

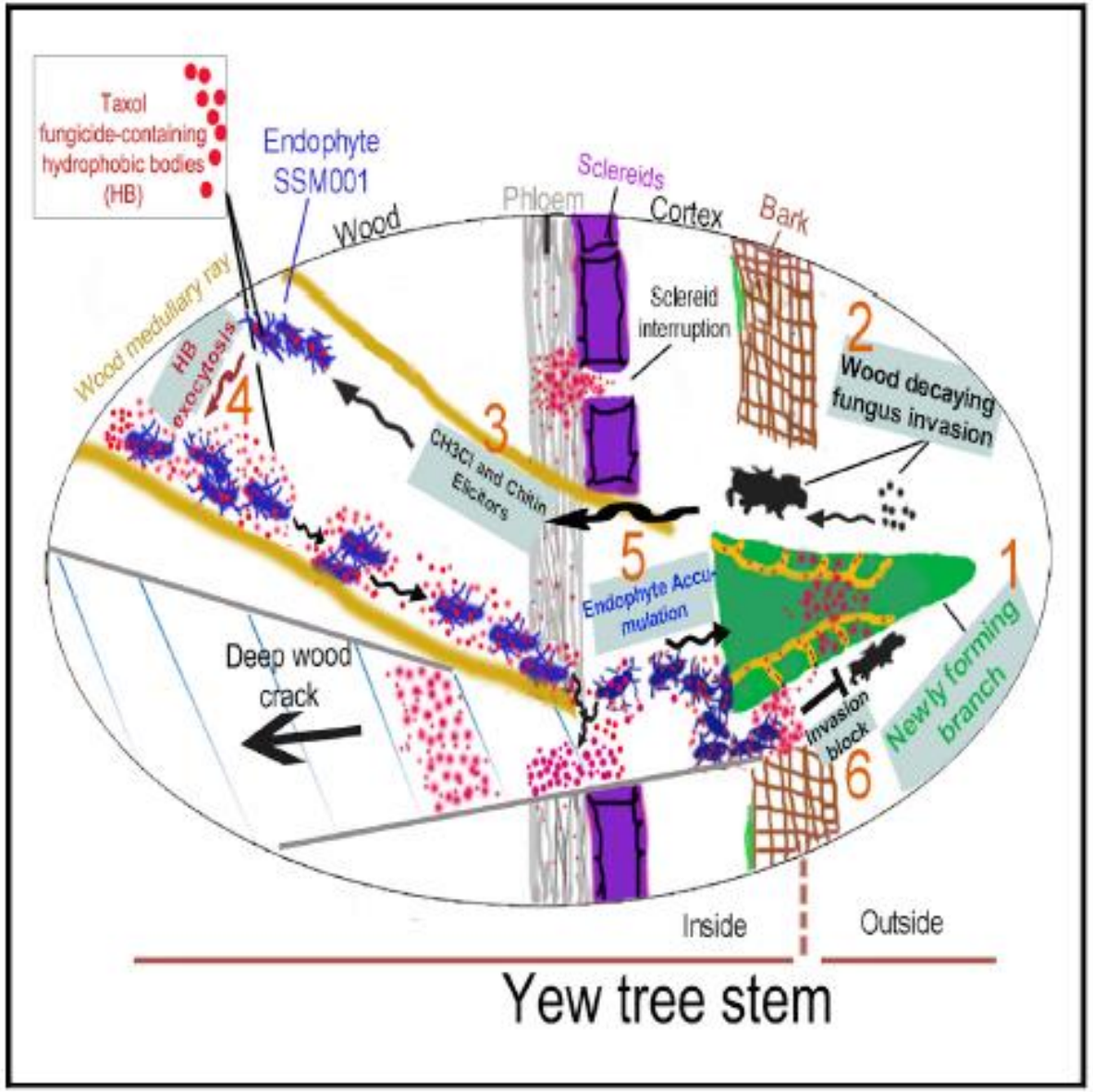
Soliman and Raizada, working with Paraconiothyrium sp. isolated from T. x media, recently obtained results in agreement with such a hypothesis . The fungus showed higher Taxol production upon treatment with the host bark extracts and more importantly, when co-cultured with one or more endophytic fungi 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

Fungal Endophytes that have Taxol Production Potential



Taxol Microbe Axis

A taxol-producing endophytic microbe that has been fully sequenced.

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.

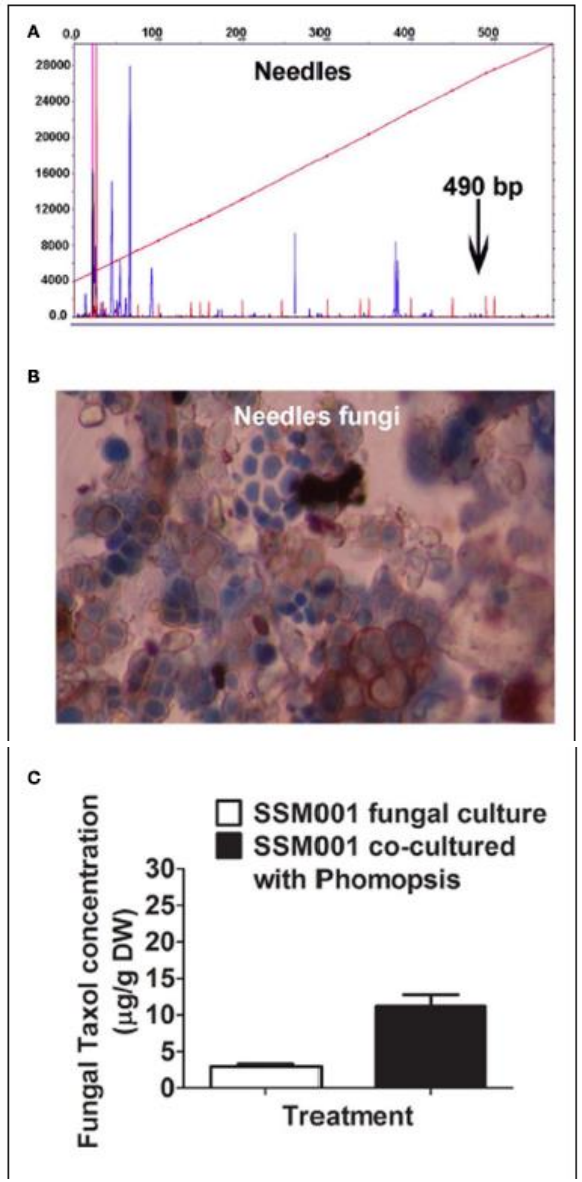
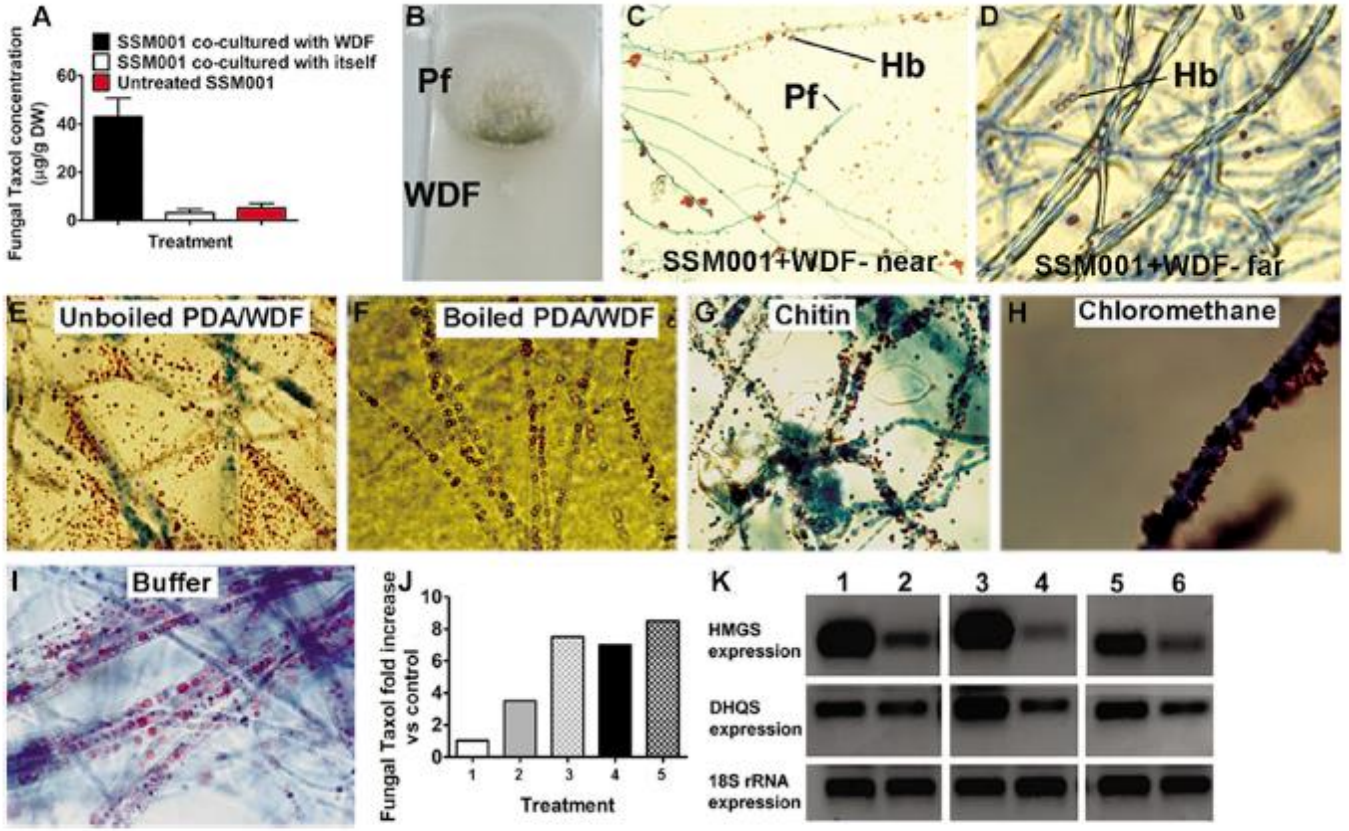


FIGURE 3 | Co-habitating fungi present in *Taxus x media* needles stimulate Taxol production from *Paraconiothyrium* SSM001 fungi. (A) tRFLP analysis of DNA pooled from *Taxus* needles showing several fungal peaks, none of which corresponded to SSM001. **(B)** Detection of different fungal spores from *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

Fungal Endophytes that have Taxol Production Potential



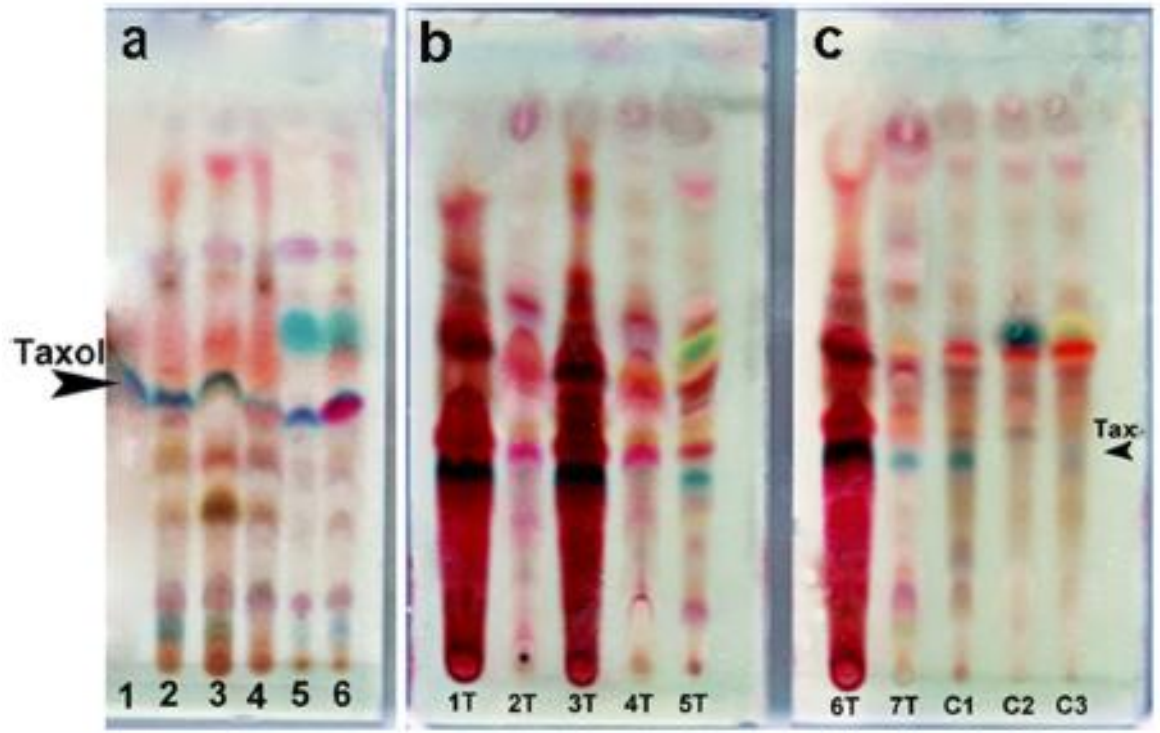
Soliman et al, Curr. Biol., 2015, 25, 2570-2576

Figure 3. Wood-Decaying Fungus and Its Diffusible Chemicals, Chloromethane and Chitin, Induce Taxol Biosynthetic Gene Expression and the Release of Taxol-Containing Hydrophobic Bodies from *Paraconiothyrium* SSM001

(J) Quantification of fungal Taxol in liquid cultures of *Paraconiothyrium* SSM001 treated with either chloromethane (8 or 16 mM), chitin (166 or 332 mg/l), or solvent methanol. In lane 1, SSM001-treated solvent control; in lane 2, SSM001-treated chitin at 166 mg/l; in lane 3, SSM001-treated chitin at 332 mg/l; in lane 4, SSM001-treated CH_3Cl at 8 mM; and in lane 5, SSM001-treated CH_3Cl at 16 mM. For additional details and controls, see Figures S3E–S3H.

Fungal Endophytes that have Taxol Production Potential

fungal terpenoid production. a Detection of Taxol on TLC from different original SSM001 strains prior to transformation process. b, c Identification of fungal Taxol in seven selected SSM001 pTFG-transformants compared to three different negative control: SSM001 fungus not treated with *Agrobacterium* (C1), SSM001 treated with wild-type *Agrobacterium* (C2), and SSM001 transformed with *Agrobacterium* harboring only hygromycin-resistant gene (C3).



Soliman et al, Appl. Microbiol, Biotech. 2017, 101, 7523-7533

Fungal Endophytes that have Taxol Production Potential

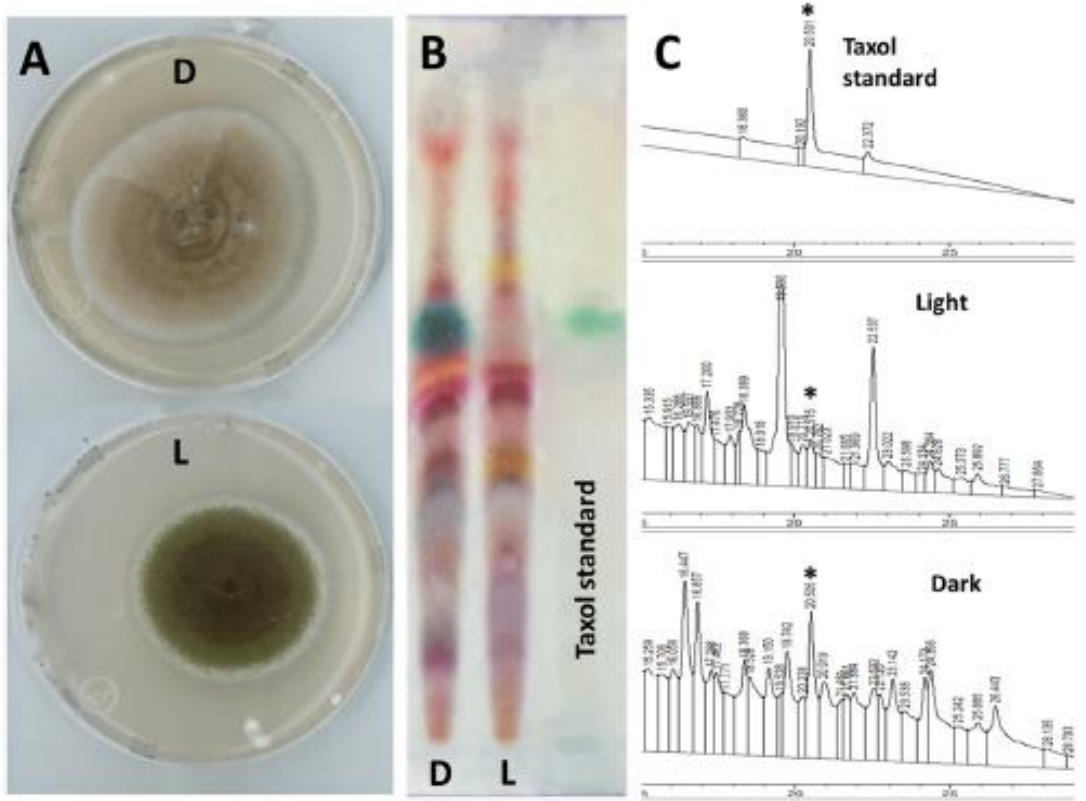


FIGURE 1 | Light pre-incubation inhibited fungal Taxol production. **(A)** Growth of Taxol-producing endophyte SSM001 fungus in light (L) and darkness (D) on PDA at 25°C for 1 week. **(B)** Detection of extracted fungal Taxol after inoculation for 3 weeks in liquid YPD broth on TLC-silica plates (10 μL sample, developing system chloroform: methanol; 5:0.5 and visualized using 0.5% vanillin/sulfuric acid reagent). **(C)** Detection and quantification of fungal Taxol by HPLC-UV when fortified with 5 ng standard Taxol. The peak area of each sample (10 μL injection volume) was measured at 233 nm. The quantification data display the mean of three replicates. The asterisk is the diagnostic peak of Taxol.

Soliman & Raizada, Front. Micro., 2018, 9, 353

Some Relevant Comments on Fungal Taxol II

Based on gene clustering in the producing fungi, it appears probable that the intact fungal pathway evolved initially in a particular way and was transmitted to other fungi and then to select plants in the genus Taxus, doing so on a geographically diverse basis.

Therefore, the extant view of plant origin first, fungal origin second, may well need to be reversed, at least in this instance.

It will be interesting to observe the evolutionary relationships between the genomic data for the paclitaxel clusters from the different endophytes derived from diverse global locations.

Daly & Cordell. JNP. 2021, 84, 871-897

Some Relevant Comments on Fungal Taxol III

Some major "points re taxol" from Daly & Cordell (2021)

- *initial yields of 1 from an individual endophyte can be significantly increased, particularly through media modification.*

- *One example is the increase in yield of 1 from Fusarium mairei from 20 to 225.2 µg/mL through modifications in nitrogen sources and using Plackett–Burman design.*

- *Endophytes producing paclitaxel (1) in culture have been isolated from 30 genera of plants in 26 plant families.*

- *Table 1 in the paper has over 100 identified fungal endophytes that produce taxol at some level.*

Daly & Cordell. JNP. 2021, 84, 871-897

Taxadiene synthase from Bacteria; Link to Cometabolism?

- *An interesting hypothesis as to why Taxadiene Synthase, the rate limiting step in taxol production appears to be "MIA" in some fungal isolates is shown in the next slide.*
- *Bacillus koreensis & Stenotrophomonas maltophilia isolated from plants with fungi that produce taxol.*
- *Could there be "cometabolism and/or linked metabolism between bacterial production of TDS and subsequent link to the fungal metabolism?*
- *Such links are known for other production of complex natural products where you have fungal/bacterial interplay in the production of Rhizoxin.*

El-Sayed et al., Sci. Pharm., 2021, 89, 48

Taxadiene synthase from Bacteria

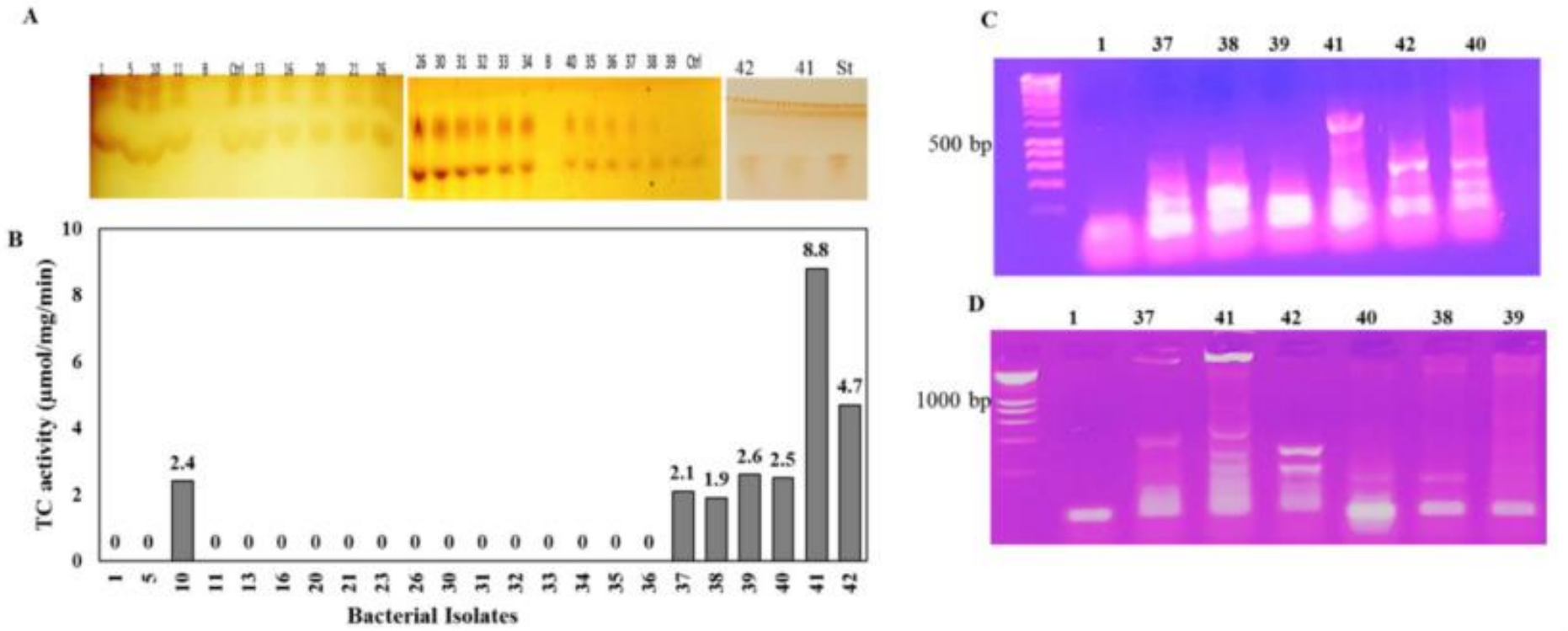


Figure 1. Screening for TDS production from different bacteria isolates.

The TLC chromatogram of residual GGPP (A) and the putative TDS activity (B) is shown. PCR amplification of tds from the selected bacterial producers using the active-site conservative primers TDS1 (C) and TDS2 (D) is shown.

El-Sayed et al., Sci. Pharm., 2021, 89, 48

Camptothecin (Mainly Current Data)

Precursors & Camptothecin Yields

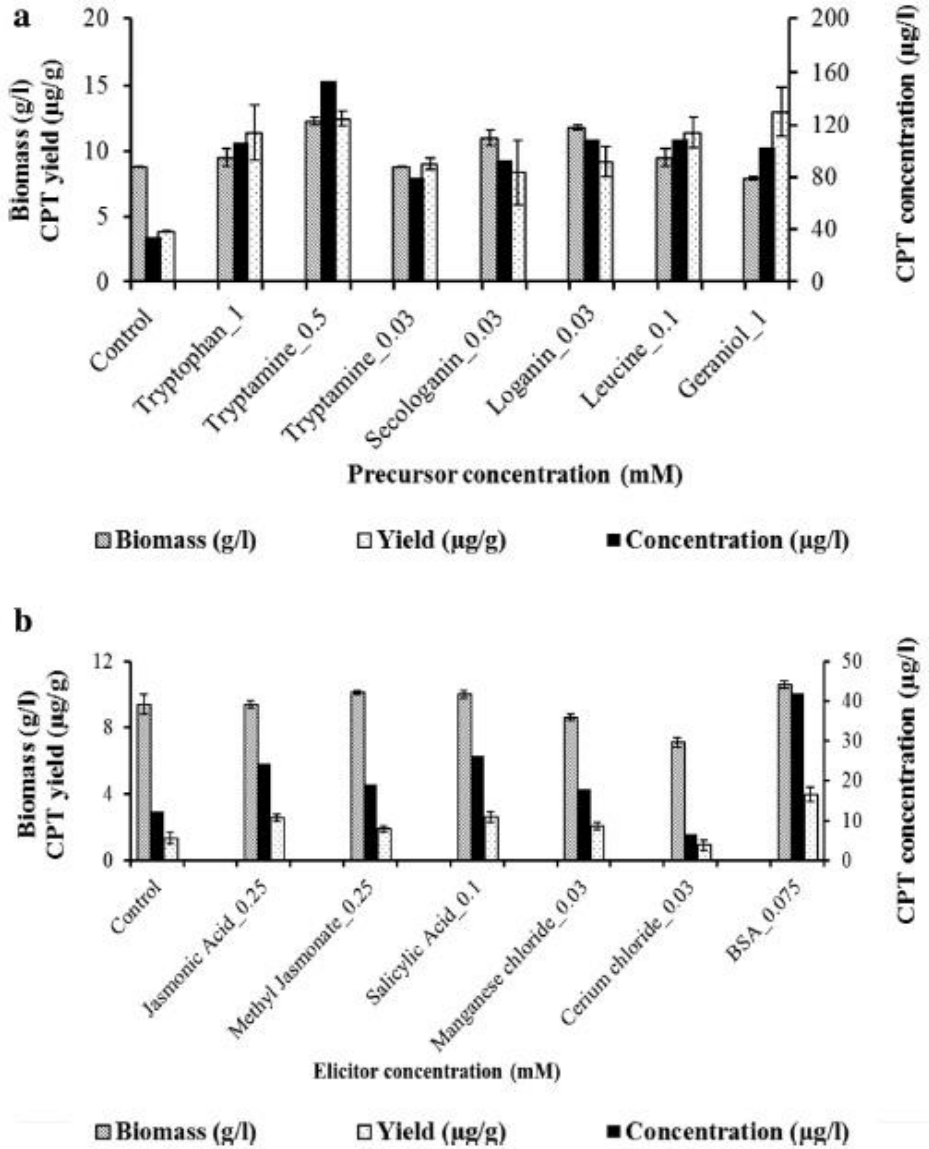


Fig. 3. (a) Effect of addition of precursors on biomass and CPT production. (b) Effect of addition of elicitors on biomass and CPT production.

Venugopalan et al, Biores. Tech., 2016, 206, 104-111

Loss of Camptothecin Yields on Subculture

Table 4 CPT production over successive generations

Subculture generation	CPT[a] (mg l^{-1})
First	283 ± 0.27
Second	198 ± 0.12
Third	102 ± 0.87
Fourth	46 ± 0.54
Fifth	0.138 ± 0.24
Sixth	0.260 ± 0.12
Seventh	0.056 ± 0.18
Eighth	0.033 ± 0.16

[a] Values of CPT are mean of five replications; S.E. calculated by GraphPad InStat3 software.

RSM = Response Surface Methodology

Bhalkar et al, RSC Adv., 2015, 5, 62828

Table 2 Experimental design of RSM studies by using four variables with six center points showing observed and predicted value for CPT production

					CPT (μg l^{-1})	
Std Order	*A*	*B*	*C*	*D*	Experimental	Predicted
1	25	60	5	1.5	28	28.82
2	35	60	5	1.5	0	−0.5166
3	25	80	5	1.5	48	47.85
4	35	80	5	1.5	1.9	2.3875
5	25	60	7	1.5	12.1	12.466
6	35	60	7	1.5	13.9	14.154
7	25	80	7	1.5	15	15.77
8	35	80	7	1.5	1	1.33
9	25	60	5	2.5	24.2	23.4
10	35	60	5	2.5	16.2	17.187
11	25	80	5	2.5	20	21.5041
12	35	80	5	2.5	0	−0.833
13	25	60	7	2.5	21.6	22.97
14	35	60	7	2.5	48	47.683
15	25	80	7	2.5	5.2	5.25
16	35	80	7	2.5	13	13.937
17	20	70	6	2	21.9	20.629
18	40	70	6	2	0	−0.02
19	30	50	6	2	14.2	13.8125
20	30	90	6	2	0	−0.9041
21	30	70	4	2	0.5	0.3958
22	30	70	8	2	0	−1.187
23	30	70	6	1	48.9	48.3625
24	30	70	6	3	56.3	55.545
25	30	70	6	2	282.2	283.2
26	30	70	6	2	282.7	283.2
27	30	70	6	2	283.1	283.2
28	30	70	6	2	283.5	283.2
29	30	70	6	2	284	283.2
30	30	70	6	2	283.7	283.2

Mixed Endophyte Cultures & CPT Levels

In any plant "system" endophytes are not "alone" they are part of a consortium that communicate constantly. In a nice elegant experiment, Bhalkar et al reported in 2016, the effect of co-culturing two endophytes from the N. nimmoniana plant that produces camptothecin. Coculture of C. fructicola & C. cassiicola gave the following levels of CPT.

Bhalkar et al, Fung. Biol., 2016, 120, 873-883

Camptothecin from mixed Fungal Cultures

Table 1 – Mono-cultures and mixed fermentations of isolated fungi.

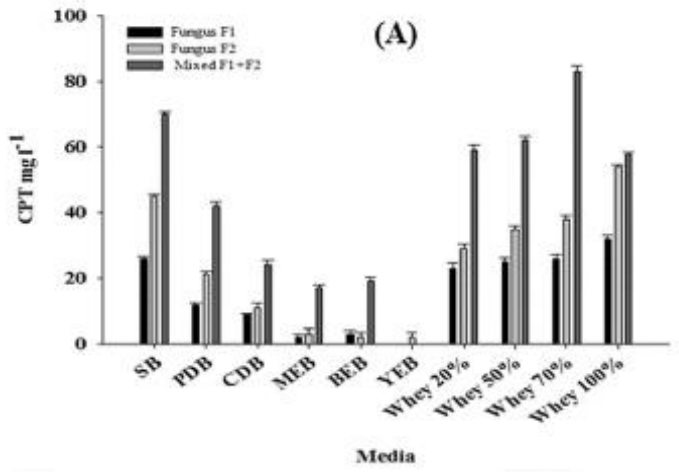
Organism (isolated fungi)	CPT detection by HPTLC	CPT quantification by HPLC (mg l^{-1})	CPT structure confirmation by LCMS (m/z)
Isolate 1 (*Fusarium oxysporum*)	Detected	90	349.1
Isolate 2 (*Fusarium* spp.)	Detected (scarcely)	ND	ND
Isolate 3	Not detected	ND	ND
Isolate 4	Not detected	ND	ND
Isolate 5 (F1)	Detected	35	349.1
Isolate 6 (F2)	Detected	70	349.1
Mixed fermentations			
Isolate 1 + Isolate 5	Detected	32	348.8
Isolate 1 + Isolate 6	Detected	45	349.1
Isolate 1 + Isolate 5 + Isolate 6	Detected	91	349.1
Isolate 5 + Isolate 6	Detected	145	349.1

ND – Not defined.

Table 2 – Classical optimization of parameters to achieve maximum CPT yield by monocultures and mixed fermentation.

Parameter studied	Fungus F1	Fungus F2	Mixed fermentation F1 + F2
Media type	Whey	Whey	Whey
Concentration of whey (%)	100	100	70
Temperature (°C)	30	35	30
pH (units)	6	6	6
Incubation period (d)	15	20	7
Agitation rate (rpm)	100	100	100
Optimized CPT yield (mg l^{-1})[a]	33	69	146

a Values denote average of three experimental data.



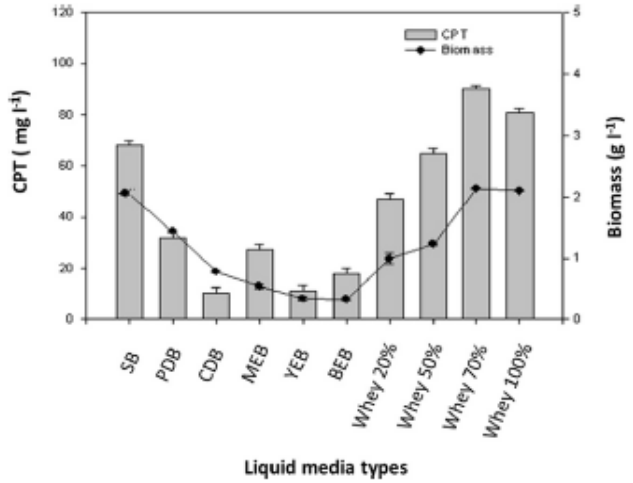


Fig 2 – Effect of different media on camptothecine and cell biomass production by mixed fermentation.

Bhalkar et al, Fung. Biol., 2016, 120, 873-883

Stability / Yield CPT

S. no	Explant	Endophytes	Dried biomass obtained from suspension cultures (g/l)	Camptothecin yield (µg/g)	Species full name	Genbank accession number	NCIM catalogue number
1	Petiole	P4-6-PE2	6.2 ± 0.1	426.7 ± 33.6	*Alternaria alstroemeriae*	MN795638	NCIM1408
2	Leaf	P4-4-LE2	8.9 ± 0.1	403.3 ± 41.6	*Alternaria burnsii*	MN795639	NCIM1409
3	Leaf	P4-1-LE1	8.5 ± 0.6	269.4 ± 53.9	*Alternaria alstroemeriae*	MN795640	NCIM1441
4	Leaf	P5-4-LE1	9.8 ± 0.2	62.5 ± 0.6	*Alternaria angustiovoidea*	MN795641	NCIM1442

Table 2. Highest yielding endophytes with their camptothecin yield, accession and deposition details.

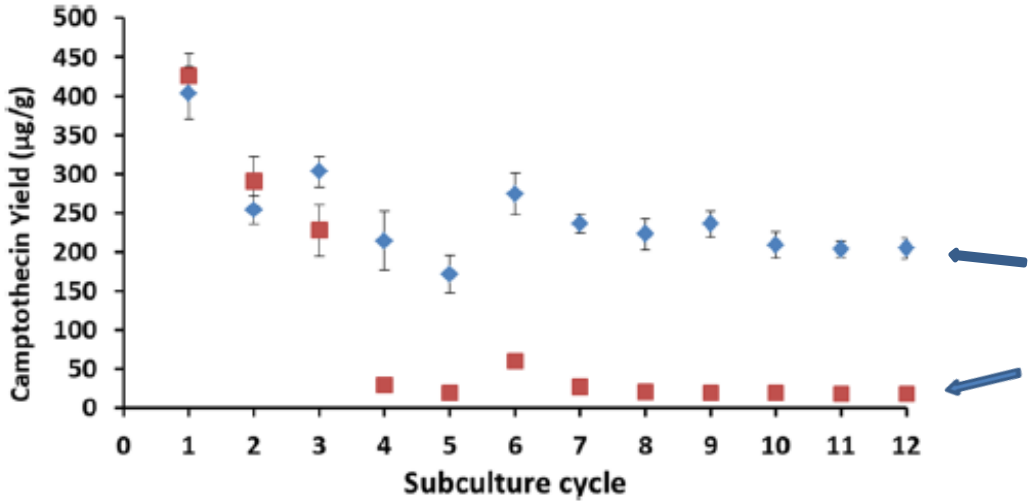


Figure 2. Stability analysis on camptothecin yield (average yield ± SEM) from the two high yielding Endophytic strains

A. burnsii NCIM1409 [blue]

A. alstroemeriae NCIM1408 [red]).

Mohinudeen et al, Sci. Rept., 2021, 11, 1478

Increasing Microbial Yield Camptothecin (2021)

Table 2

Different strategies used to increase the production of camptothecin in endophytes. There are two calculation methods for the production of camptothecin in endophytes: μg/g refers to the content of camptothecin in the dry weight of endophytes, and μg/L refers to the content of camptothecin in the fermentation broth.

Strain	Yield	Container	Strategy used	References
E. infrequens	0.496 ± 0.073 μg/g	Bioreactor	Bioreactor vs Shake flask	Amna et al. (2006)
Nodulisporium sp.	45 μg/g	Bioreactor	Bioreactor vs Shake flask	Rehman et al. (2009)
Xylaria sp.	5400 μg/L	Shake flask	Eliciter	Liu et al. (2010)
E. infrequens	12.50 μg/g	Shake flasks	Sabouraud with tryptophan and leucine	Amna et al. (2012)
T. atroviride	98.52–142.15 μg/L	Shake flask	Optimized fermentation conditions, elicitor, and adsorbent resin	Pu et al. (2013)
Fusarium oxysporum	2.83×10^5 μg/L	Shake flask	Response surface methodology	Bhalkar et al. (2015)
B. rhodina	71830 μg/g	Shake flask	DNA methyltransferase inhibitors	Vasanthakumari et al. (2015)
F. solani	22.6 μg/L	Shake flask	Potato dextrose medium with 2% (v/v) of ethanol	Venugopalan and Srivastava (2015)
F. solani	40.2 μg/L	Bioreactor	Potato dextrose medium with 5% (v/v) of ethanol	Venugopalan and Srivastava (2015)
F. oxysporum	0.61 μg/g	Shake flask	Response surface methodology	Musavi et al. (2015)
C. fructicola and C. cassiicola	1.46×10^5 μg/L	Shake flasks	Co-culture	Bhalkar et al. (2016)
F. solani	41.9 μg/L	Shake flask	PDA(tryptamine (0.5 mM) as precursor and bovine serum albumin (BSA) (0.075 mM) as an elicitor)	Venugopalan et al. (2016)
Bacillus sp.	0.068 μg/g	Shake flask	Role of plasmid	Soujanya et al. (2017)

Ruan et al, Food Chem. Tox., 2021, 151, 112113

Camptothecin & Plasmid Presence

Table 2
Morphological and molecular characterization of endophytic bacterial isolates from *Pyrenacantha volubilis* along with their CPT content.

Plant part used for isolation	Bacterial morphology (from gram staining)	Endophytic bacterial identity			GenBank Accession number	CPT content µg/g[a]
		Species	16s RNA sequence match (%)	Homology		
Fruit	Positive rods	*Bacillus* sp.	97	JN089354.1	KP125955	0.09
Fruit	Positive rods	*Bacillus subtilis*	97	HQ834723.1	KY741853	0.106
Fruit	Positive rods	*Bacillus amyloliquefaciens*	99	KM378584.1	KY741854	0.028
Leaf	Positive rods	*Bacillus* sp.	95	HM142601.1	KP125956	0.068

[a] CPT was determined at the end of 3rd day of incubation.

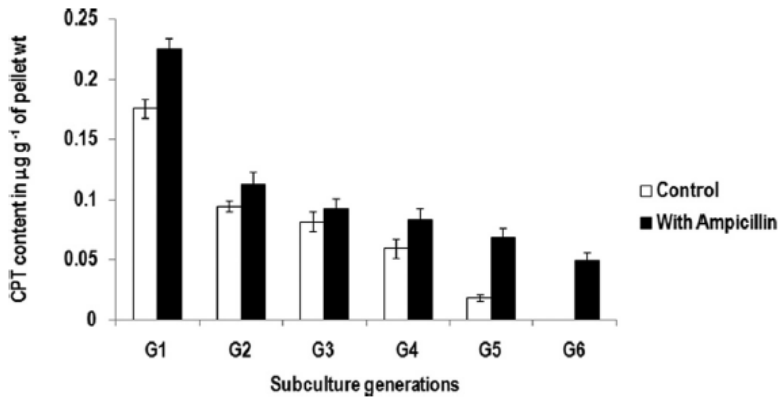


Fig. 4. CPT (µg g^{-1} pellet) of isolate, *B. subtilis* (KY741853) sub-cultured in ampicillin containing media (in open) and in control without ampicillin (in bold). Bars indicate standard deviation (SD) ± over three replications. G1 to G6 indicate sub-culture generations.

Ampicillin resistance implied presence of a plasmid.

Treatment with acriflavine removed all plasmid/Cpt yield.

Addition of plasmid to "cured" microbe produced Cpt.

Soujanya et al, Phytomed., 2017, 36, 160-167

Current Information on Camptothecin Sources

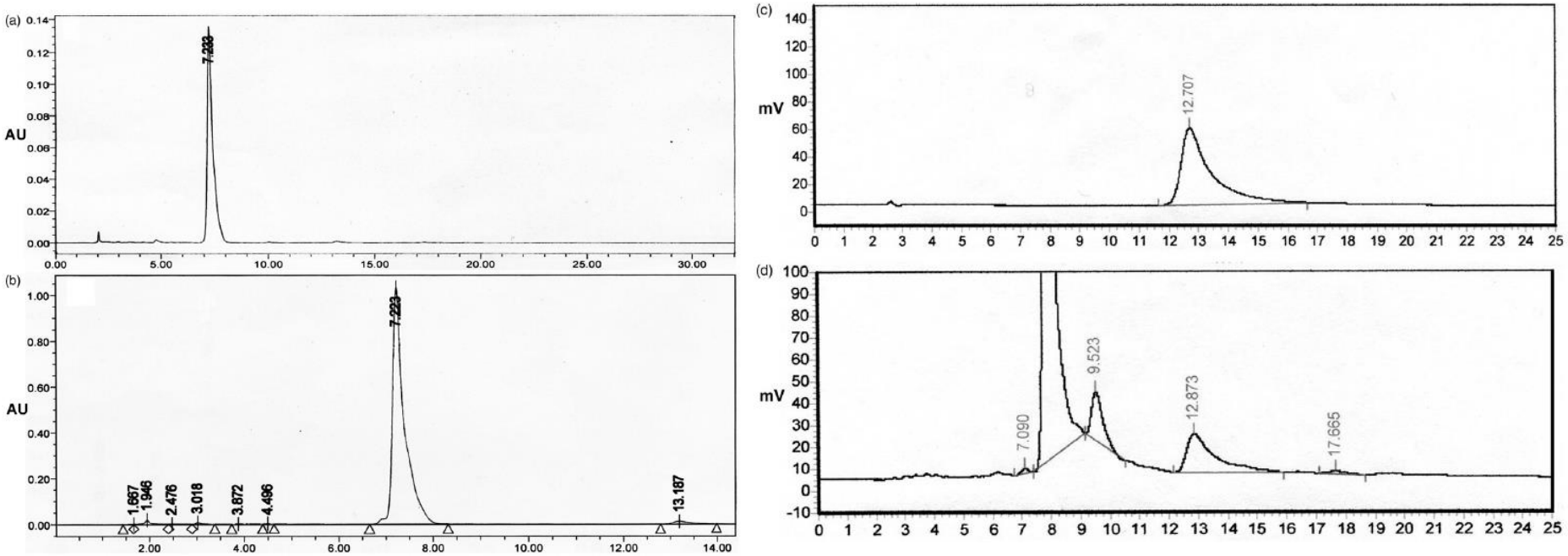
Table 2
Endophytic production of CPT in different host plants.

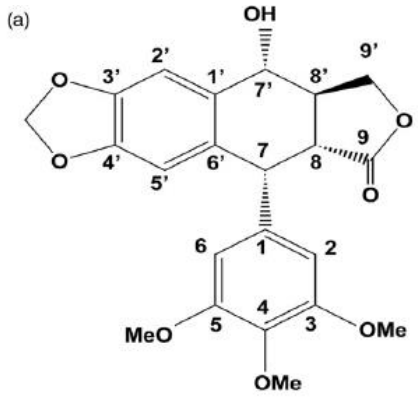
Sl. no.	Host plants	Families	Type(s) of endophytes	Endophytic organisms	Analytical methods	References
1	*Chonemorpha fragrans* (Moon) Alston	Apocynaceae	Both fungal and bacterial strains	–	RP-HPLC and ESI–Q-TOF	Clarance et al., 2019a
2	*Piper betel* L.	Piperaceae	Fungal strains	*Aspergillus niger*	HPLC	Aswini and Soundhari, 2018
3	*Pyrenacantha volubilis* Hook.	Icacinanceae	Bacterial strains	*Bacillus* sp. (KP125955 and KP125956), *Bacillus subtilis* (KY741853) and *Bacillus* sp. (KY741854)	ESI-MS/MS and NMR	Soujanya et al., 2017
4	*Camptotheca acuminata*	Nyssaceae	Fungal strain	*Fusarium solani* (S-019)	TLC, HPLC and EI-MS	Ran et al., 2017
5	*Nothapodytes nimmoniana*	Icacinaceae	Fungal strain	*Colletotrichm fructicola* (SUK1) and *Corynespora cassiicola* (SUK2)	HPLC and LC-MS/MS	Bhalkar et al., 2016
6	*Camptotheca acuminata*	Nyssaceae	Bacterial strain	*Paenibacillus polymyxa* (LY214)	HPLC-DAD	Pu et al., 2015
7	*Camptotheca acuminata*	Nyssaceae	Fungal strains	*Fusarium nematophilum* (XSXY09), *Alternaria alternata* (XSQZ04) and *Phomopsis vaccinii* (XSCY02)	HPLC	Su et al., 2014
8	*Camptotheca acuminata*	Nyssaceae	Fungal strains	*Aspergillus* sp. (LY341), *Aspergillus* sp. (LY355) and *Trichoderma atroviride* (LY357)	HPLC-DAD	Pu et al., 2013
9	*Miquelia dentata* Bedd.	Icacinaceae	Bacterial strains	*Bacillus subtilis* (JQ956522.1), *Bacillus* sp. (JN700911.1), *Bacillus cereus* (JF935135.1) and *Lysinibacillus* sp. (JN160728)	LC-MS and ESI-MS/MS	Shweta et al., 2013a, 2013b
			Fungal strains	*Fomitopsis* sp. (MTCC1017), *Alternaria* sp. (MTCC5477) and *Phomposis* sp.		
10	*Nothapodytes foetida* (Wight) Sleumer	Icacinaceae	Fungal strain	*Entrophospoa infrequens*	HPLC	Amna et al., 2012; 2006
11	*Camptotheca acuminata*	Nyssaceae	Fungal strain	*Fusarium solani*	HPLC and LC-ESI-HRMS	Kusari, 2009; 2011
12	*Apodytes dimidiata* E. Mey. exArn.	Icacinaceae	Fungal strains	*Fusarium solani* (MTCC 9667 and 9668)	LC-MS/MS	Shweta et al., 2010
13	*Nothapodytes nimmoniana*	Icacinaceae	Fungal strains	*Fusarium* sp., *Diaporthesp.*, *Irpex* sp., *Botryosphaea* sp. and *Galactomycessp.*	LC-MS	Gurudatt et al., 2010
14	*Nothapodytes foetida*	Icacinaceae	Fungal strain	*Neurospora* sp. (ZP5SE)	HPLC and LC-MS	Rehman et al., 2008
15	*Camptotheca acuminata*	Nyssaceae	Fungal strain	*Alternaria* sp.	HPLC	Zheng et al., 2007

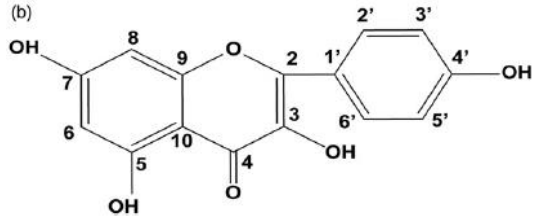
Kaur et al, S. A. J. Botany, 2020,134, 135-145

Podophyllotoxin and Homoharringtonine

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







NMR match as well

Huang et al, Pharm Biol, 2014

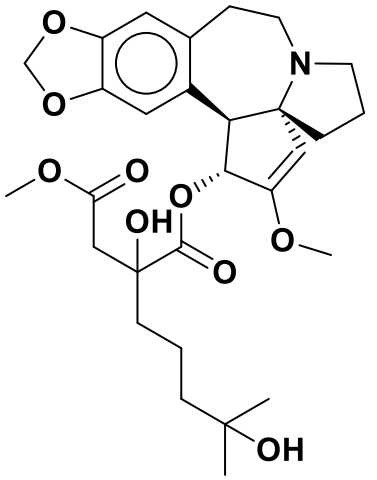
"Homoharringtonine" may well be another?

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/identification. Powell et al, Tetrahedron Lett., 1970, 11, 815-818

Maytansine

Maytansine & Root Microbes

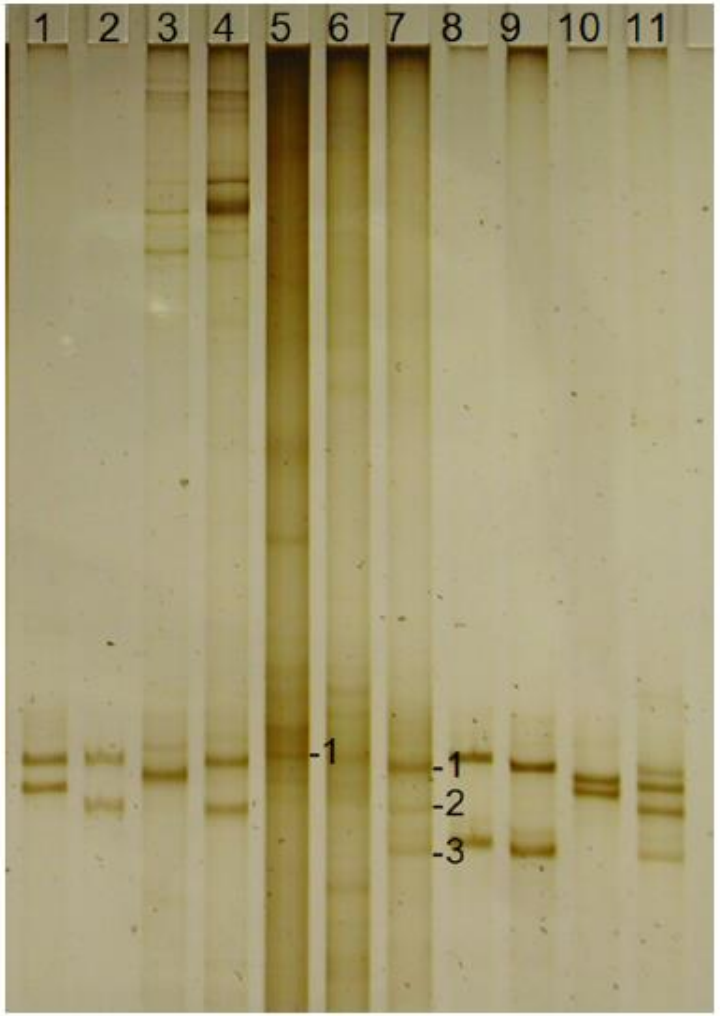
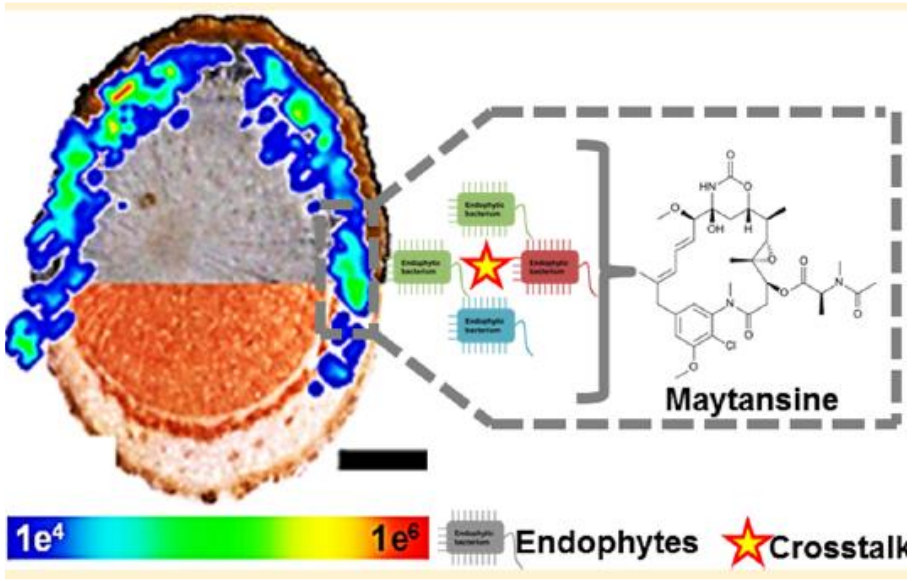


Fig. 2. SSCP gel of the V3 region of the 16S rDNA of the bacterial community in the rhizosphere of three different *P. verrucosa* plants (lane 5 and 6 of unknown maytansine content, lane 7 of known maytansine content i.e. plant FG1 also annotated plant "V" in Pullen et al., 2003). Reference strains: *Kitasatospora putterlickiae* (lane 1), *Actinosynnema mirum* (lane 2), *Actinosynnema pretiosum* ssp. *pretiosum* (lane 3), *Actinosynnema pretiosum* ssp. *auranticum* (lane 4), R12 (lane 8), 27a (lane 9), *Amycolatopsis mediterranei* (lane 10), *Actinoplanes teichomyceticus* (lane 11).

(i) a known bacterial species (in this case A. pretiosum ssp. auranticum) taken through a SSCP procedure followed by sequencing can be recovered by this technique.
(ii) a bacterium with a V3 region identical to that of A. pretiosum ssp. auranticum is present in the rhizosphere of P. verrucosa.

Wings et al, Phytochem., 2013, 91, 158-164

Maytansine & Root Microbes



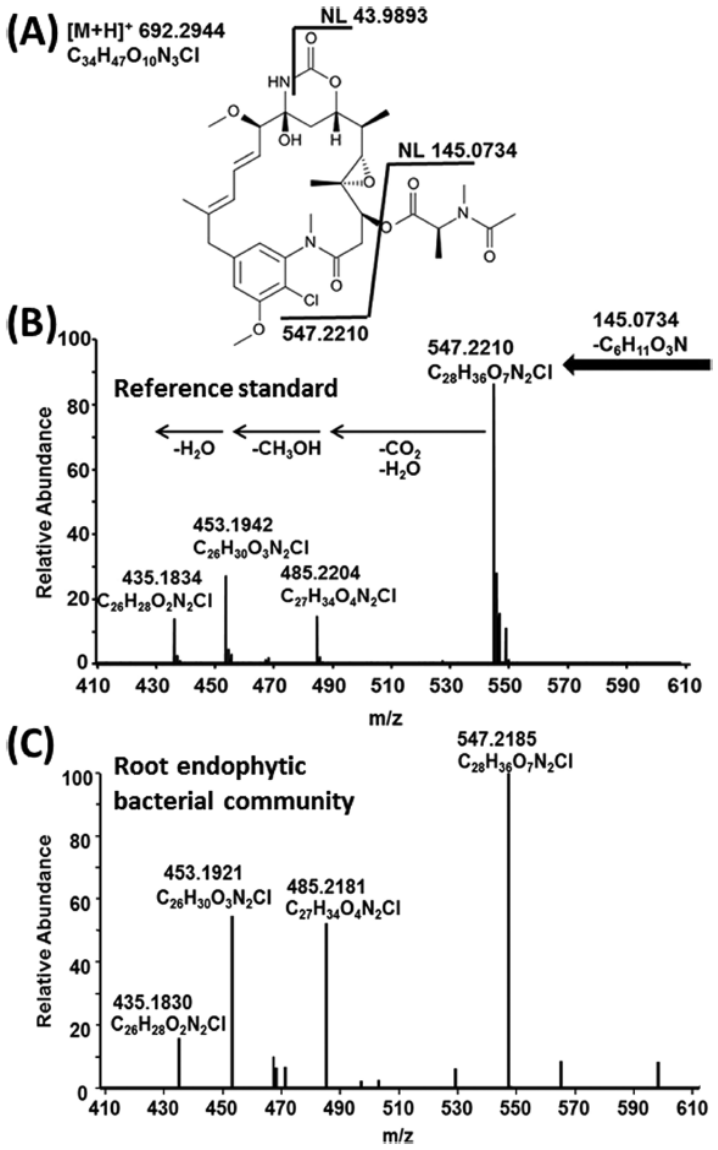


Figure 1. Structural formula and HRMS2 fragmentation of maytansine.
(A) Structure of maytansine.

(B) HRMS2 fragmentation of authentic maytansine standard.

(C) Representative HRMS2 fragmentation of maytansine produced by root endophytic bacterial community

Kusari et al, J. Nat. Prod., 2014, 77, 2577-2584

Vinca alkaloids

Isolation of Endophytes from C. roseus Leaves

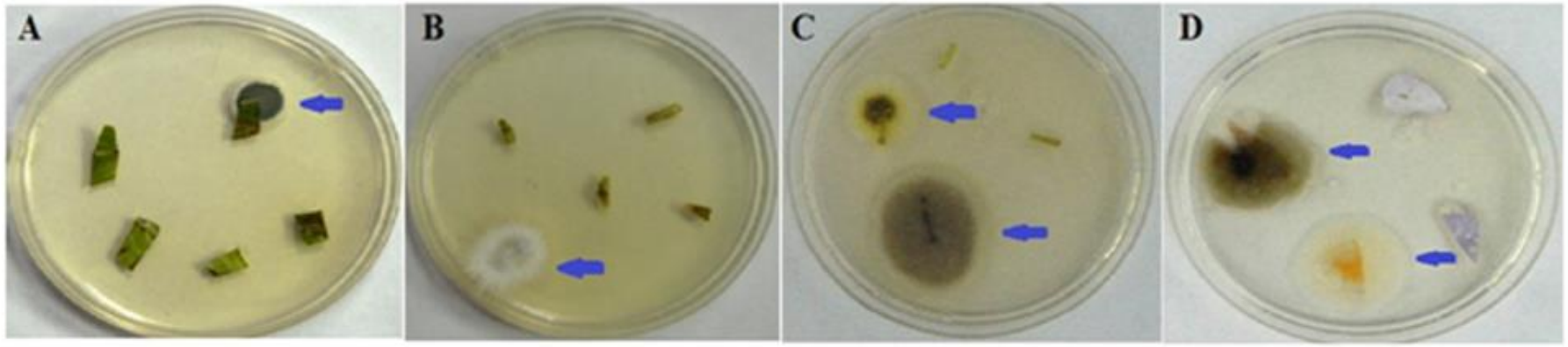


Fig 1. Isolation of endophytic fungi from *C. roseus* plant. Arrowheads indicate the emergence of endophytic fungi from the plant cuttings. A-leaf, B-stem, C-pedicel and D-flower petal.

Palem et al, PlosOne, 2015, 10, e0144476

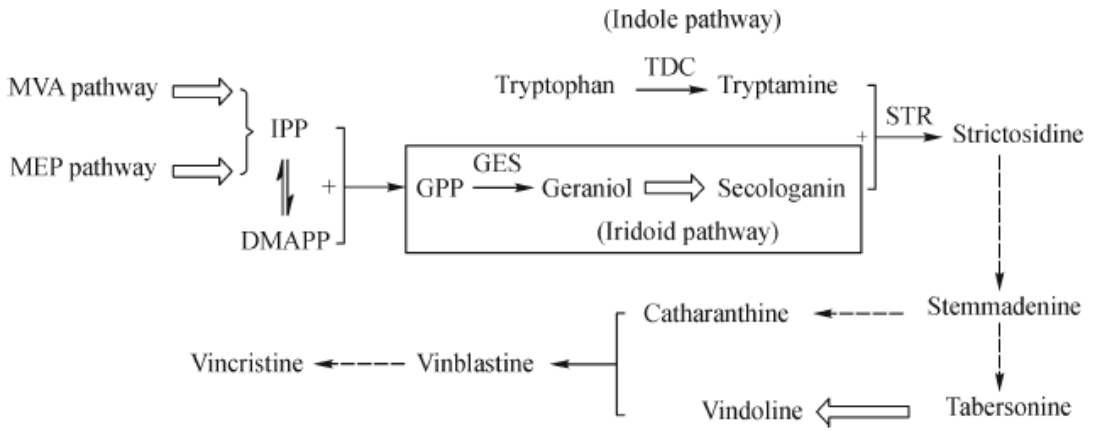


Fig. 1 An overview of the pathways leading to TIAs biosynthesis. Solid arrows indicate one-step reaction; dashed arrows indicate uncharacterized steps; white arrows indicate multi-step reactions.

Zhu et al, Front. Med., 2014, 8, 285-293

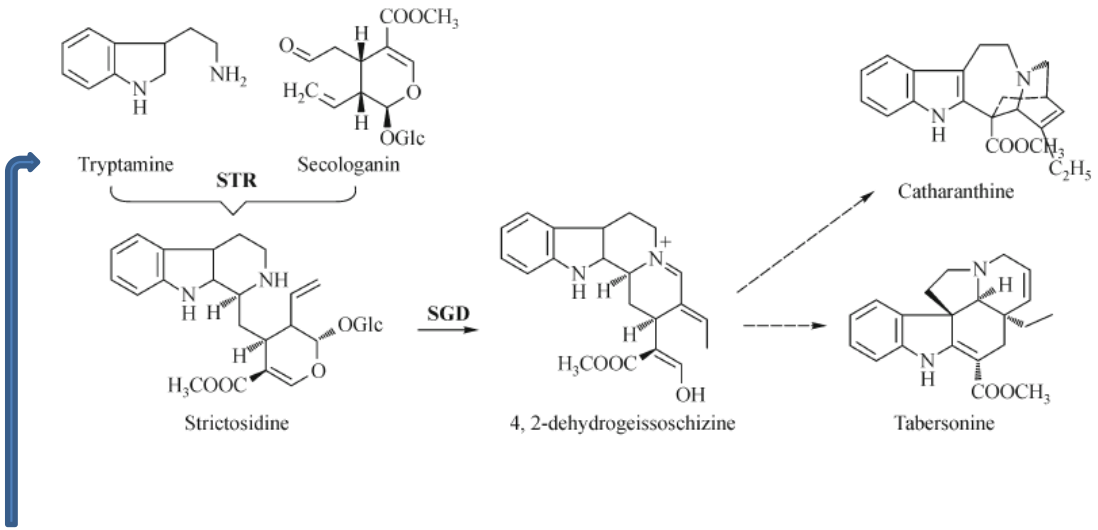
Fungi from C. roseus and their Bioactivities

Table 2. Details of the endophytic fungi isolated from *C. roseus* and the antiproliferative effects (IC_{50} values) of their crude extracts against human HeLa cells.

Fungal isolate code[a]	Identified fungi[b]	Plant organ[c]	GenBank accession numbers	Cytotoxic activity IC_{50}[d](µg/mL) of filtrate extracts	Cytotoxic activity IC_{50}[d](µg/mL) of mycelia extracts
CrP1	*Alternaria alternata*	F	KC503496	50	>100
CrP2	*Colletotrichum kahawae*	F	KC503497	>100	>100
CrP3	*Colletotrichum gloeosporioides*	F	KC503498	68.5	>100
CrP4	*Colletotrichum gloeosporioides*	F	KC920830	25	>100
CrP5	*Alternaria tenuissima*	P	KC920831	>100	100
CrP6	*Aspergillus niger*	P	KC920832	>100	>100
CrP7	*Aspergillus niger*	P	KC920833	25.5	>100
CrP8	*Colletotrichum gloeosporioides*	P	KC920834	70	>100
CrP9	*Alternaria tenuissima*	P	KC920835	47.5	92.5
CrP10	*Colletotrichum kahawae*	P	KC920836	>100	100
CrP11	*Flavodon flavus*	S	KC920837	>100	>100
CrP12	*Aspergillus niger*	S	KC920838	23	>100
CrP13	*Aspergillus niger*	S	KC920839	>100	100
CrP14	*Eutypella Species*	S	KC920840	13.5	100
CrP15	*Dothideomycetes Species*	S	KC920841	>100	>100
CrP16	*Eutypa species*	S	KC920842	>100	>100
CrP17	*Talaromyces radicus*	S	KC920843	52	46
CrP18	*Talaromyces radicus*	L	KC920844	>100	>100
CrP19	*Chaetomium globosum*	L	KC920845	77.5	>100
CrP20	*Talaromyces radicus*	L	KC920846	20	13
CrP21	*Fusarium solani*	S	KC920847	39.5	>100
CrP22	*Alternaria species*	F	KC920848	49.5	>100

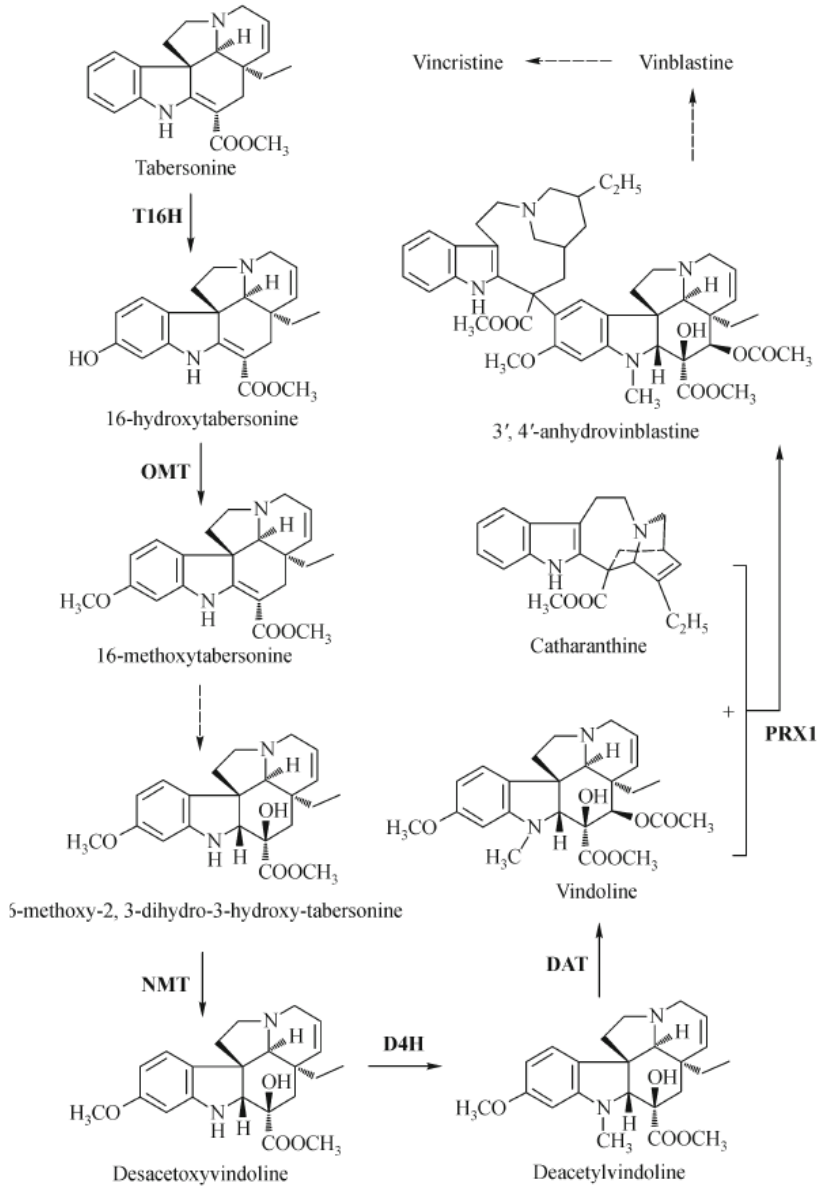
Palem et al, PlosOne, 2015, 10, e0144476

Vinca Alkaloids



Formation of tryptamine by TDC (tryptophan decarboxylase) is one of two key enzymes, the other being anthranilate synthase

Zhu et al, Front. Med., 2014, 8, 285-293



Vinca Alkaloids from Endophytes

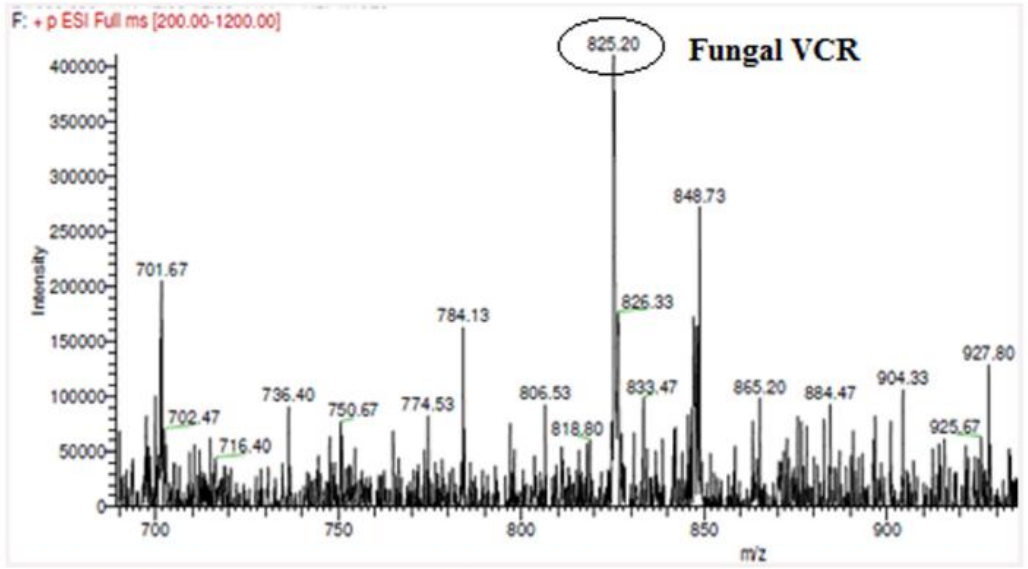


Fig 9. LC-ESI-MS analysis. of fungal VCR. The mass spectrum of the fungal extract showed a ($M+H^+$) peak at a molecular mass of 825.46, which was identical to that observed in the mass spectrum of the VCR standard.

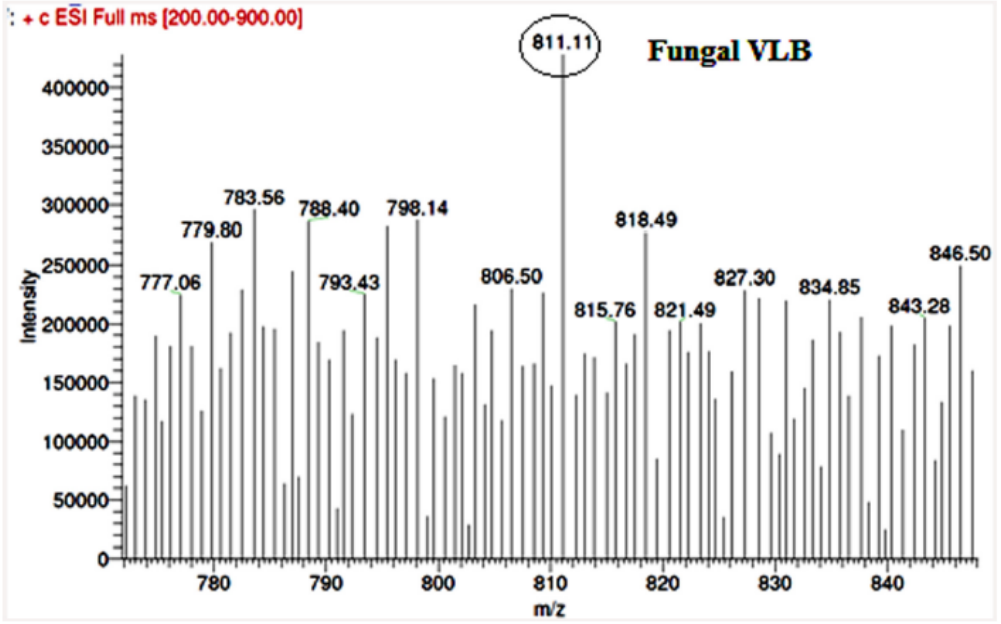
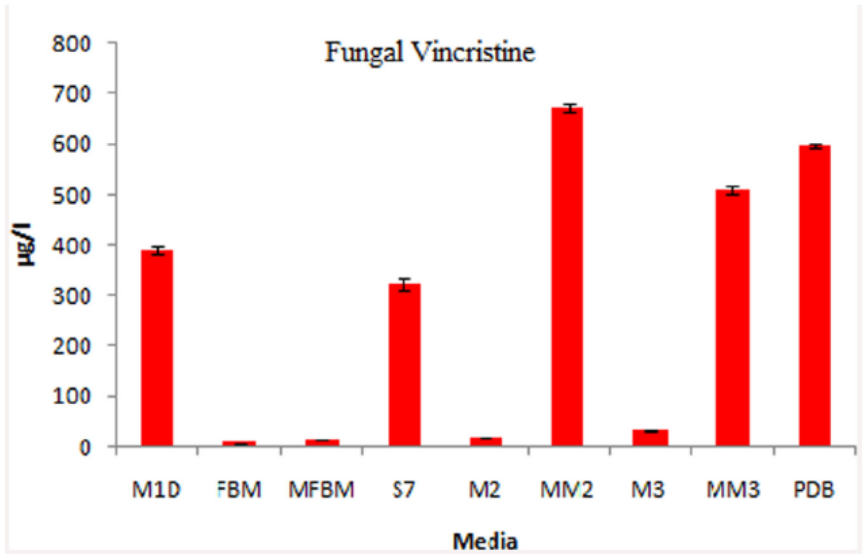
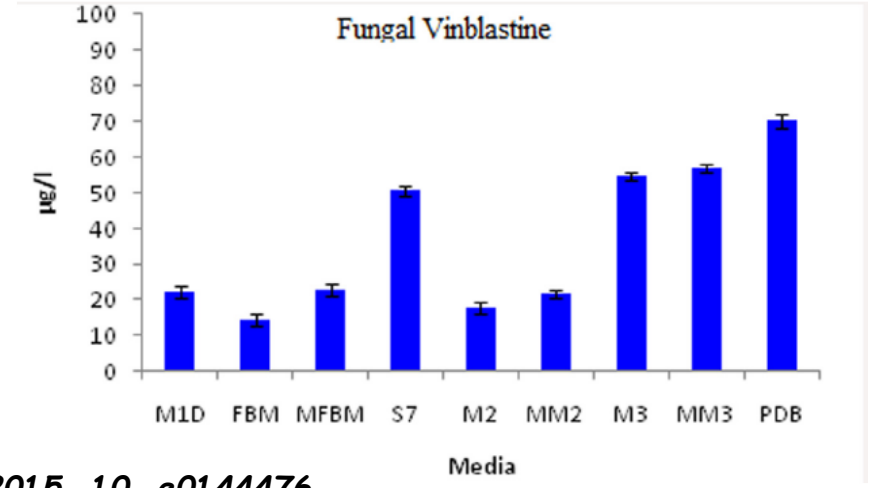


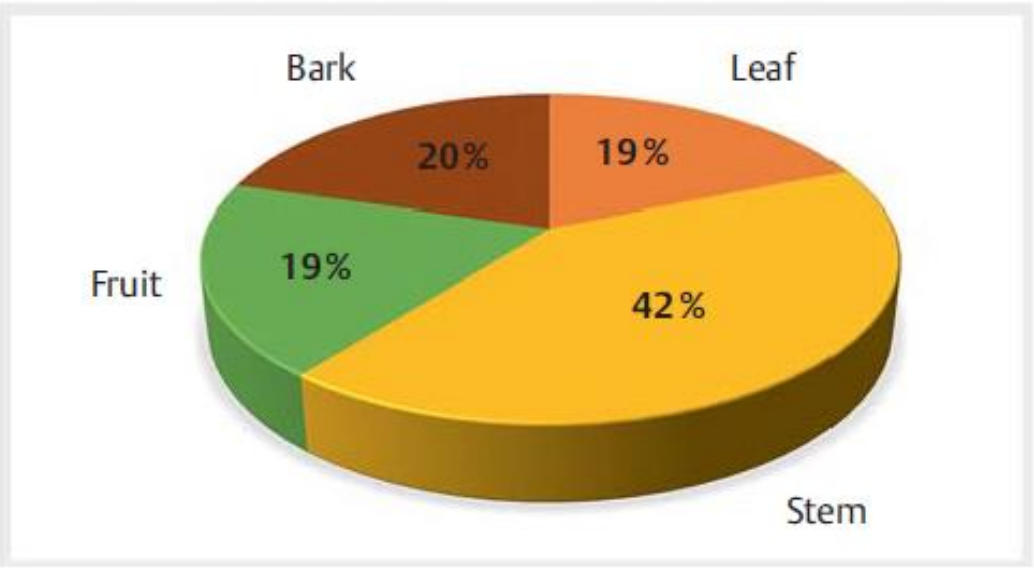
Fig 10. LC-ESI-MS analysis of fungal VBL. The mass spectrum of the fungal extract showed a ($M+H^+$) peak at a molecular mass of 811.51, which was identical to that observed in the mass spectrum of the VBL standard.



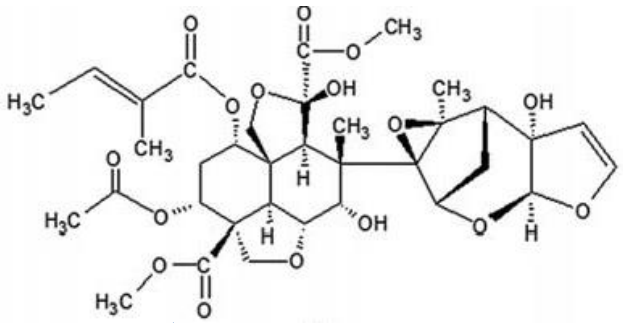


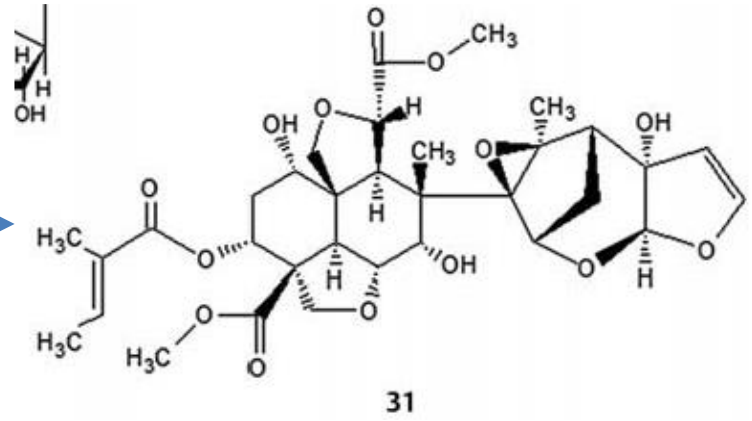
Palem et al, PlosOne, 2015, 10, e0144476

Neem



▶ **Fig. 4** Percentage of the endophytic fungi isolated from different parts of neem.





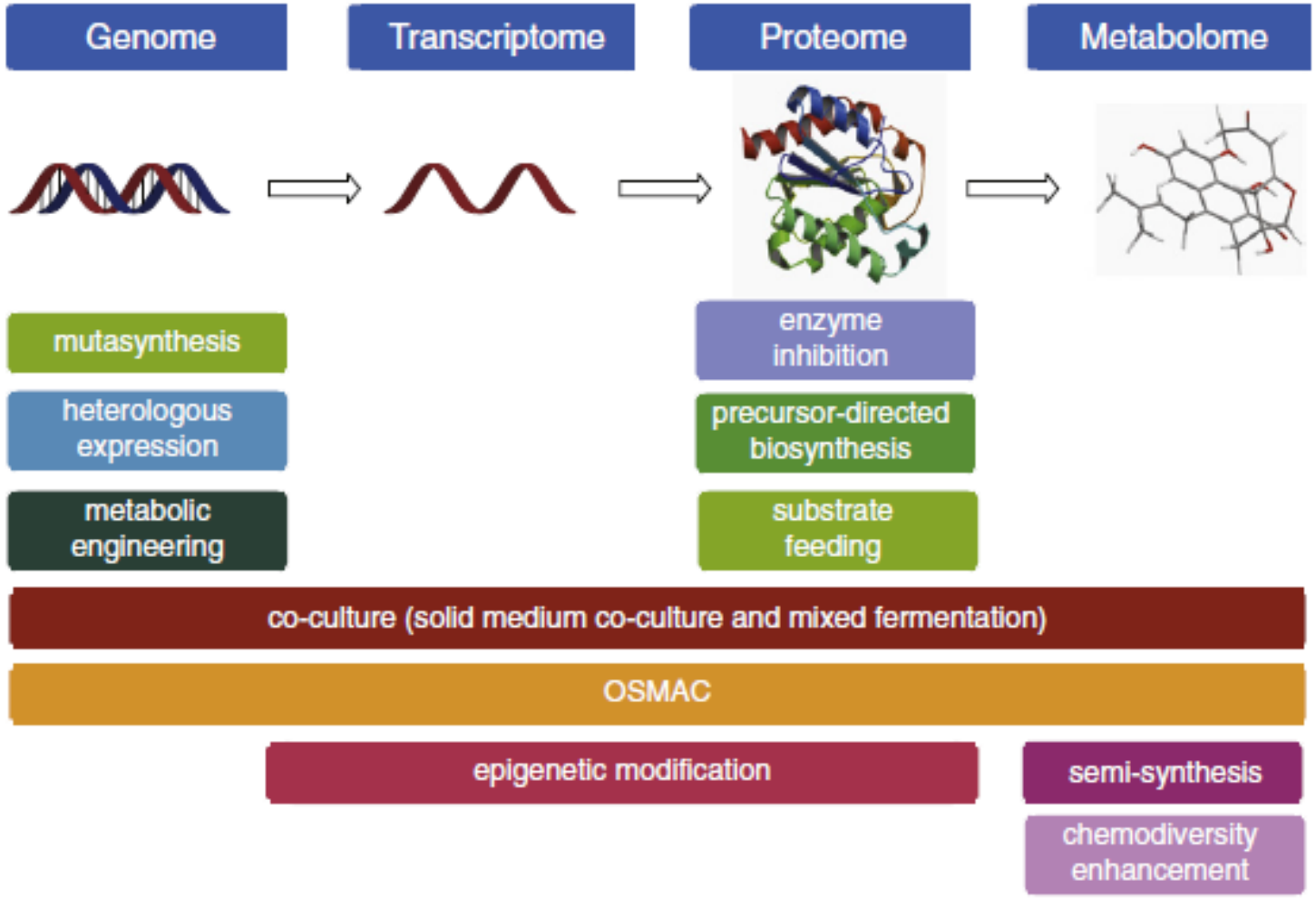
▶ **Table 2** *Continued*

Sl. No.	Compound	Derivative	Activity	Endophytic fungi	Reference
30	Azadirachtin A (**30**)	Ring-C-seco-tetrano-triterpenoids	Insecticidal activity	*Eupenicillium parvum*	[294]
31	Azadirachtin B (**31**)	Ring-C-seco-tetrano-triterpenoids	Insecticidal activity	*Eupenicillium parvum*	[294]

Kharwar et al, Planta Med., 2020, 86, 906-940

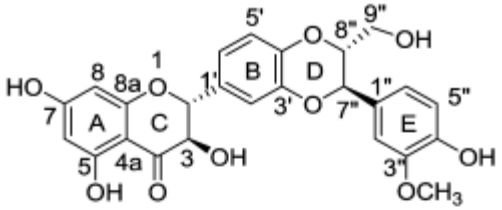
Potential Methods to Improve Microbial Yields

Potential Methods To Overcome Low Yield in Epi / Endophyte Fermentations

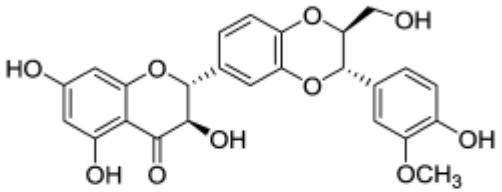


Bertrand et al, Biotech Adv 2014

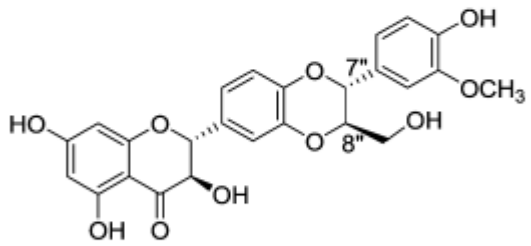
Silybin (Milk Thistle) add back "plant components"



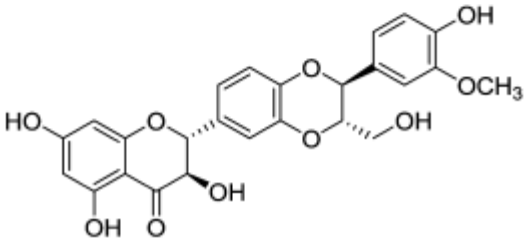
Silybin A (**1**)



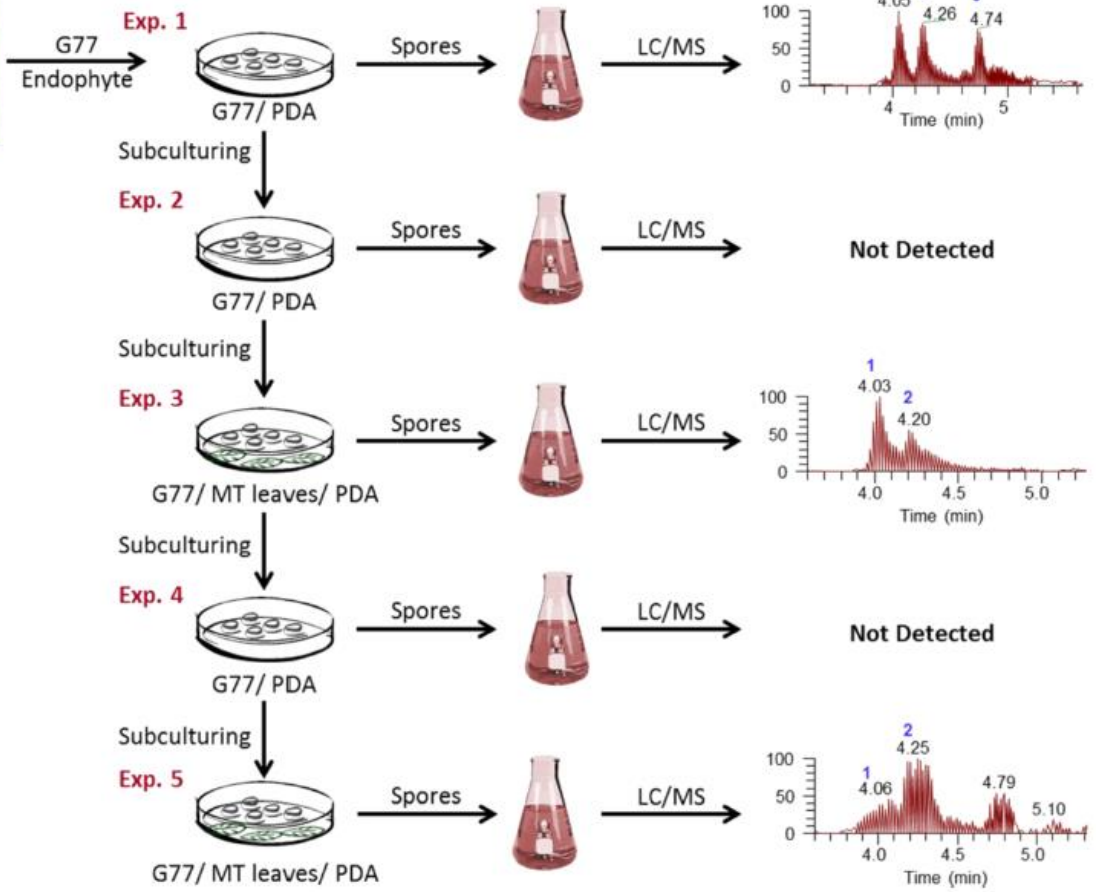
Silybin B (**2**)



Isosilybin A (**3**)



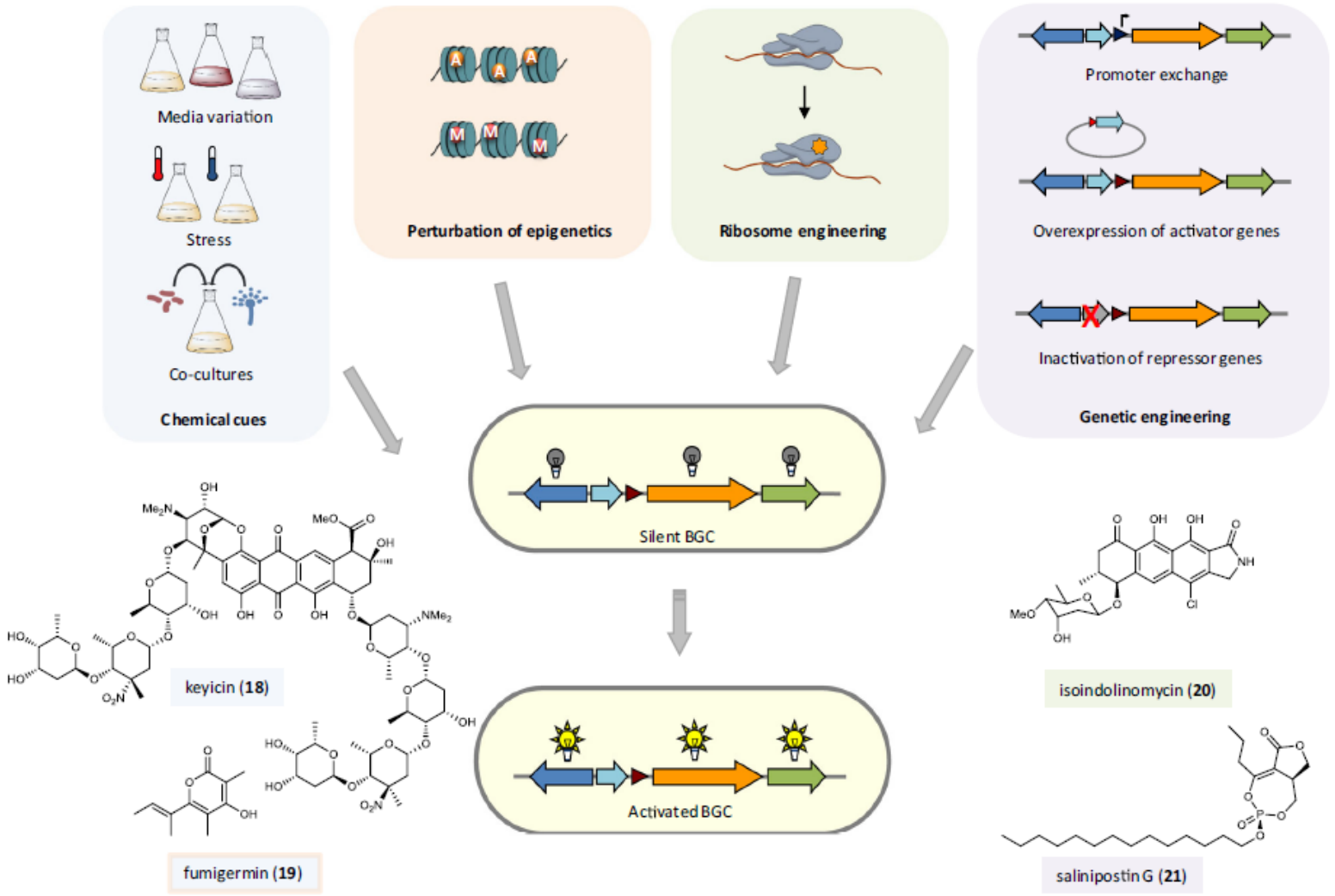
Isosilybin B (**4**)



Aspergillus iizukae

El-Elimat et al, JNP, 2014

Activation of Silent Genes



Strategies for the activation of silent biosynthetic gene clusters in native hosts and examples of natural products discovered through these methods. (BGC biosynthetic gene cluster).

Sherlach and Hertweck, Nat. Comm. 2021,12, 3864

Artificial Intelligence as a Discovery Tool

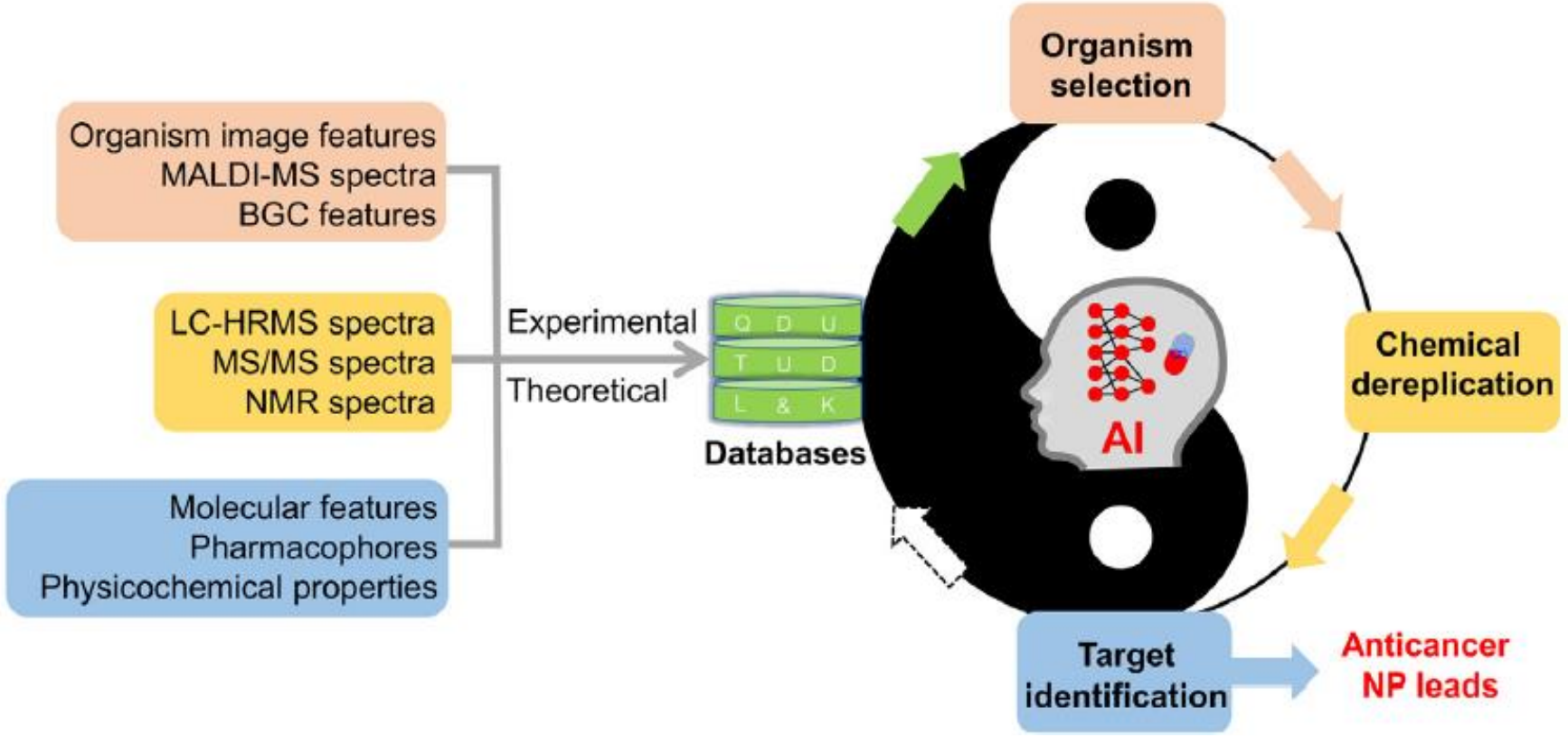


Figure 1. Artificial intelligence (AI)-guided discovery of anticancer lead compounds from plants and associated microorganisms

Li et al, Trends Cancer, 2022, 8, 65-80

Marine Endophytic Microbial Sources

Following the Marine Path !

Until roughly 1960, current dogma held that if you wanted to modify a nucleoside you could modify the base as much as you wished, but the sugars had to be either Deoxyribose or Ribose to maintain biological activity.

However, the marine sponge community did not get the MEMO!!

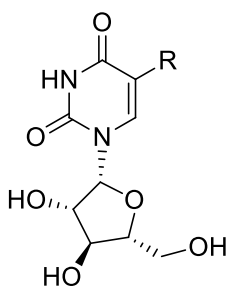
Cryptotheya crypta
now Tectitheya crypta
Bahamas

Bergmann at Yale in the very early 1950s isolated two molecules, spongothymidine and spongouridine. They contained arabinose not ribose or deoxyribose.

However, Seymour Cohen did take note and showed that what is now known as Ara-C had been synthesized by Evans at Upjohn (reported in 1961), would kill tumor cells. Later an antiviral activity was reported as well. Now known to be produced by a Vibrio

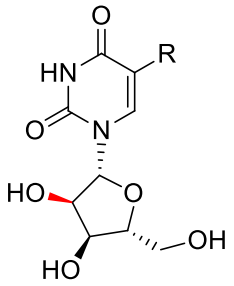
Ergo, T. crypta had not received the Memo!

Since Mother Nature Did Not Receive The Current Dogma Memo !



Spongothymidine; R = CH_3

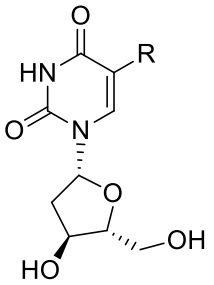
Spongouridine; R = H



Ribothymidine

Ribouridine

Sugar = Ribose

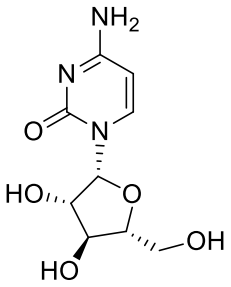


Thymidine

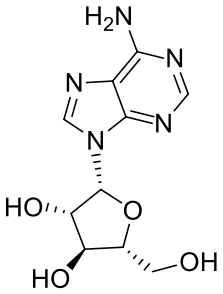
Uridine

Sugar = Deoxyribose

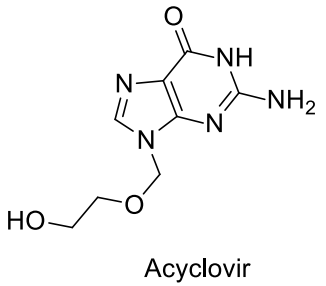
Led to



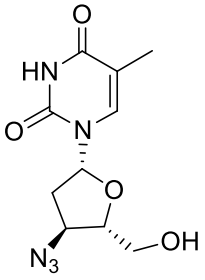
Ara-C



Ara-A



Acyclovir



AZT

Rest is history; all synthetic non-ribose/deoxy ribose nucleosides evolved from ST & SU

Diet as Defense

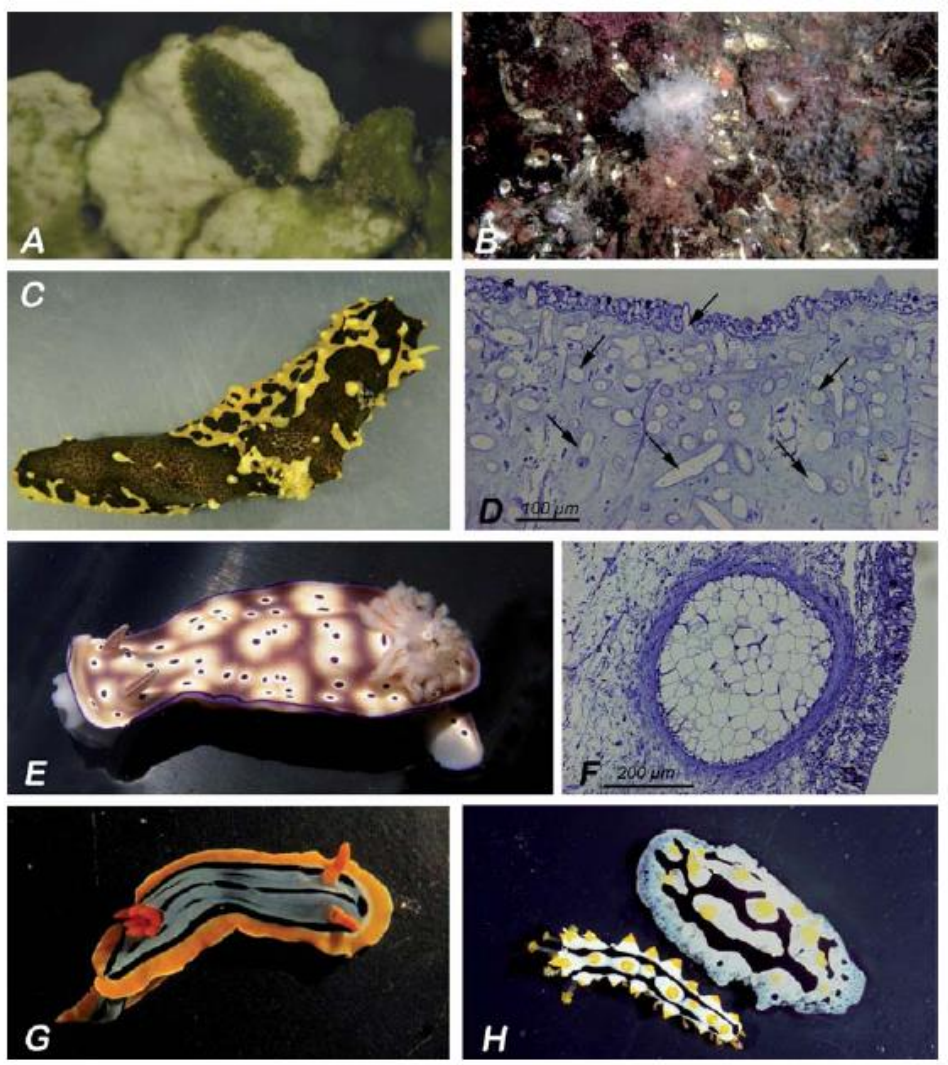


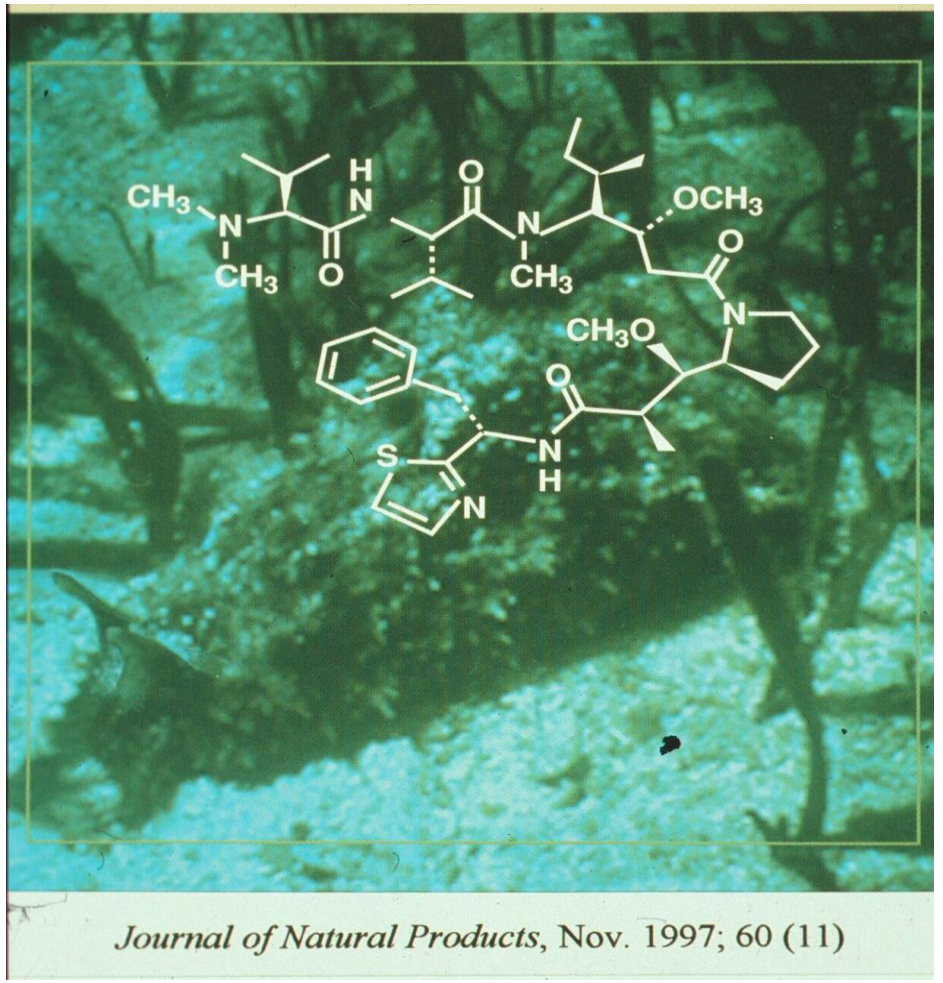
Fig. 2 Defensive mechanisms in opisthobranchs. A. Specimen of the sacoglossan *Elysia pusilla* on its food organism *Halimeda* (Lizard Island, Australia). B. Two specimens of *Dendronotus frondosus*, a white and a red form, in their natural habitat (Kungsfjord, Sweden, depth 10 m); size of animals about 3 cm. C. *Notodoris gardineri* (Lizard Island, Australia), a doridoidean feeding on sponges. D. Cross-section of notum (*Notodoris citrina*) showing many spicules in the tissue (arrows). E. *Risbecia tryoni* (Lizard Island, Australia); size of animal about 3 cm. F. *Risbecia tryoni*, mantle dermal formation in which natural products are usually stored. G. *Chromodoris elizabethina* (Lizard Island, Australia); size of animal about 2 cm; animal possesses mantle dermal formations with toxic compounds. H. *Phyllidia varicosa*, a sponge feeder known to incorporate toxic compounds. The warning colours of the slug are mimicked by juveniles of the holothurian *Pearsonathuria graeffei*.

Many examples where the sources are

Now known to be from the diets

Cyanobacteria in these cases

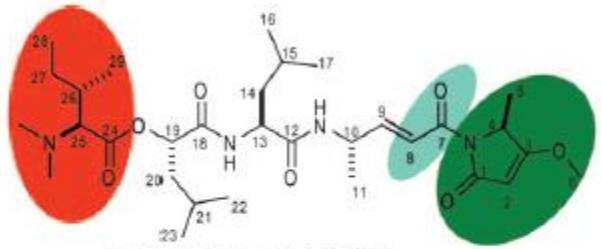
"You are what you eat"



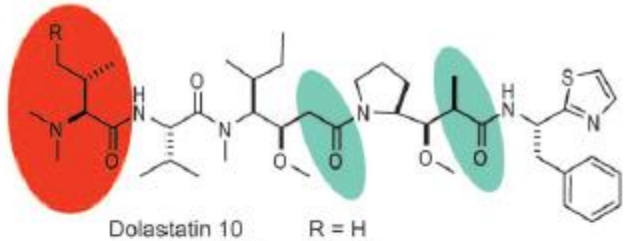
Dolabella auricularia

Dolastatins come from a Symploca species that they graze on

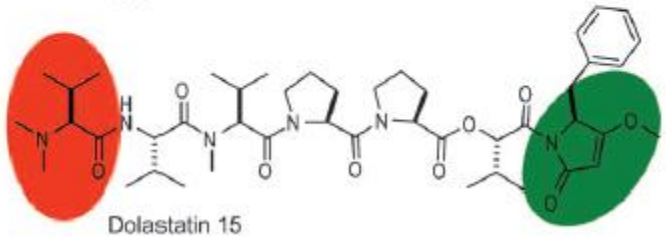
Mother Nature's Peptide Combichem



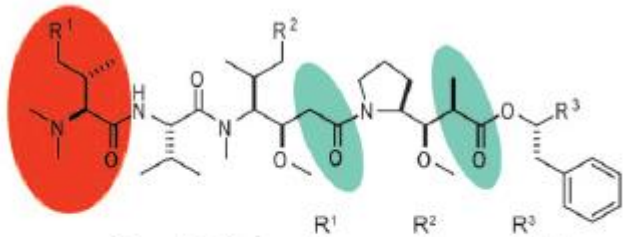
Symplostatin 4 (1) 25*S*,26*S*
Gallinamide A 25*R*/*S*,26*R*/*S*



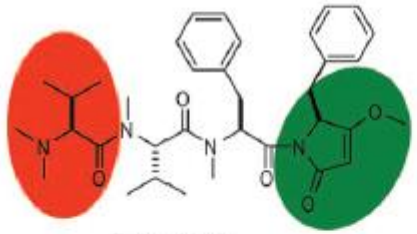
Dolastatin 10 R = H
Symplostatin 1 R = Me



Dolastatin 15



	R^1	R^2	R^3
Symplostatin 3	H	Me	CO_2H
Malevamide D	Me	H	CH_2OH

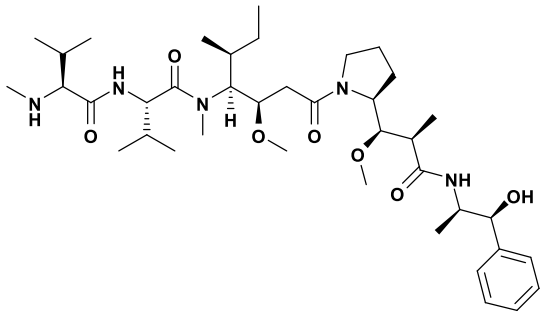


Belamide A

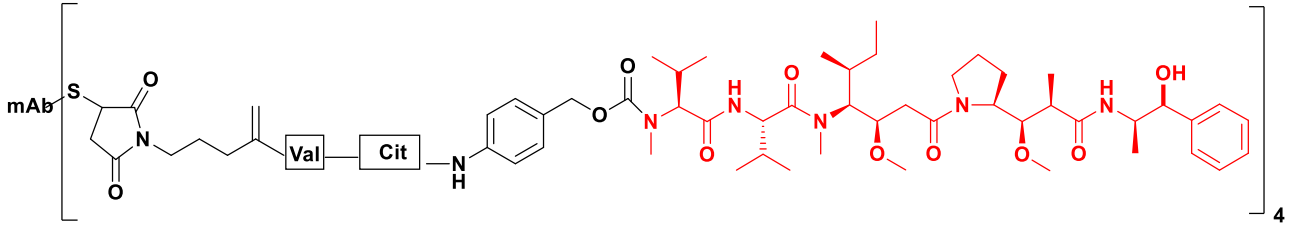
Figure 1. Structures of dolastatin 10, its cyanobacterial analogues symplostatins 1 and 3 and malevamide D, as well as dolastatin 15, its truncated analogue belamide A and the hybrid-like symplostatin 4 (**1**) and gallinamide A. All compounds except dolastatin 15 have been isolated from cyanobacteria. Characteristic features for each compound are indicated.

Taori et al CBC 2009

Immunoconjugate-linked Auristatin PE Derivative



Vedotin



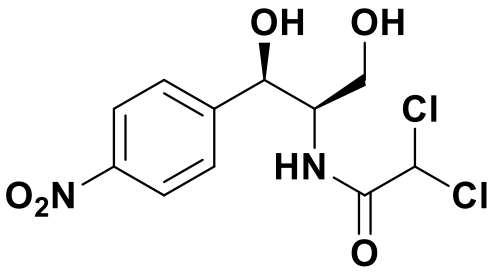
101; Brentuximab vedotin; Red color is monomethylauristatin E

N-terminus linked to a Mab is now approved and many variants are in Phase I, II & III linked to different Mabs (over 30 in trials)

Many variations such as monomethylauristatin F (different C-terminus)

Marine Metabolites I

By way of introduction, in the Marine area, though the systematic investigation from a secondary metabolite aspect, only really occurred in the last 45 or so years, it became apparent early on that bioactive compounds, or close relatives that were known from terrestrial sources, were being reported.

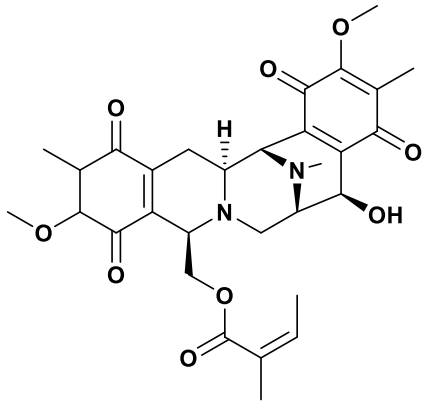


Chloramphenicol

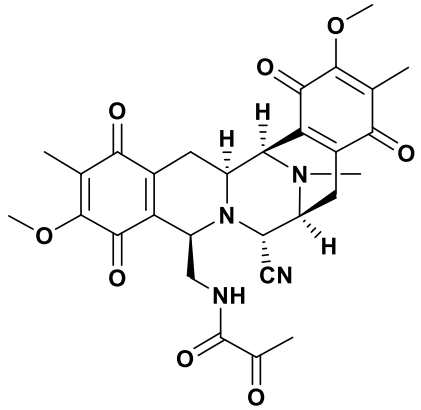
Price et al, JA, 1981

Marine Metabolites II

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



1. Renieramycin A

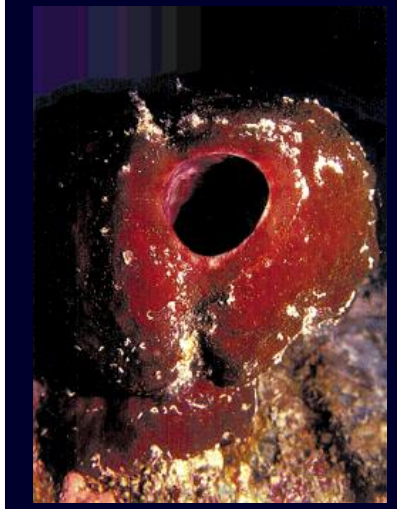


2. Saframycin A

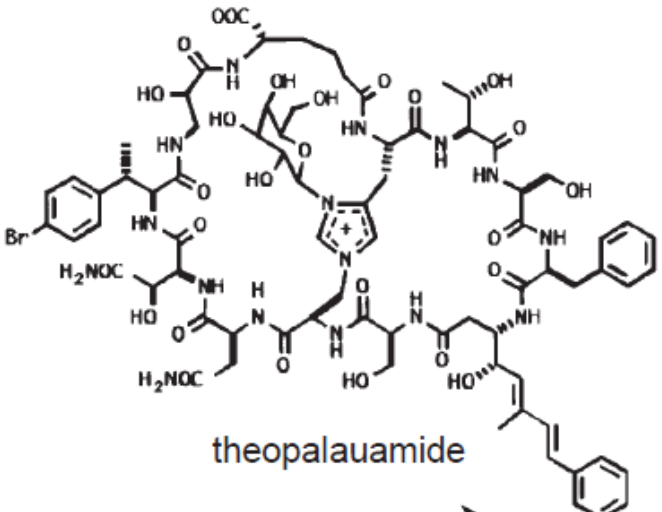
The saframycins had been reported as antibiotics by Arai in 1977 from the terrestrial microbe S. lavendulae, and as antitumor agents in 1980.

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

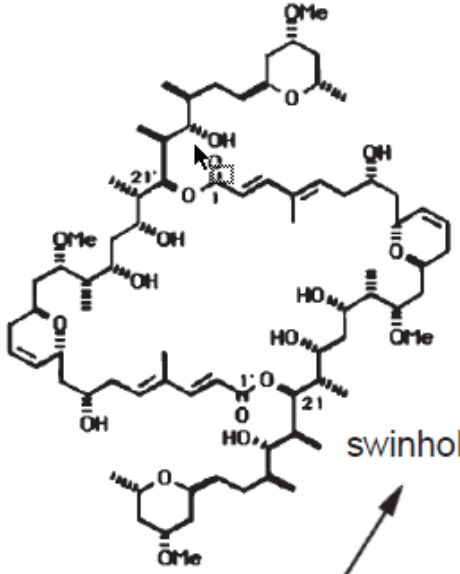
Early Evidence for Cryptic Microbial Production



Paluan chemotype of
Theonella swinhoei

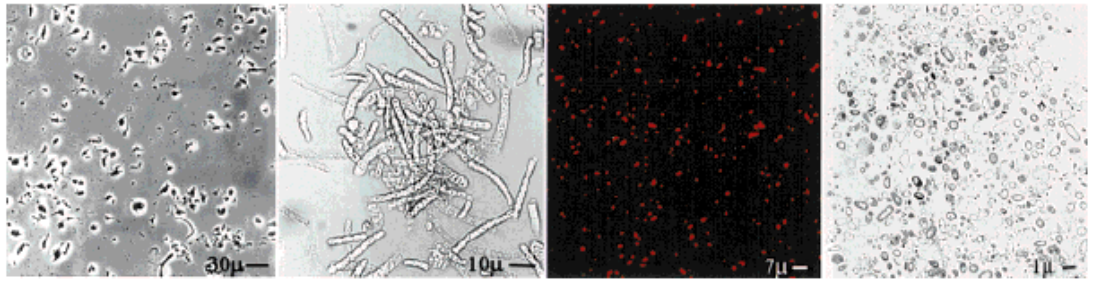


theopalauamide



swinholide A

Micrographs of purified cell types. Associated chemistry shown above

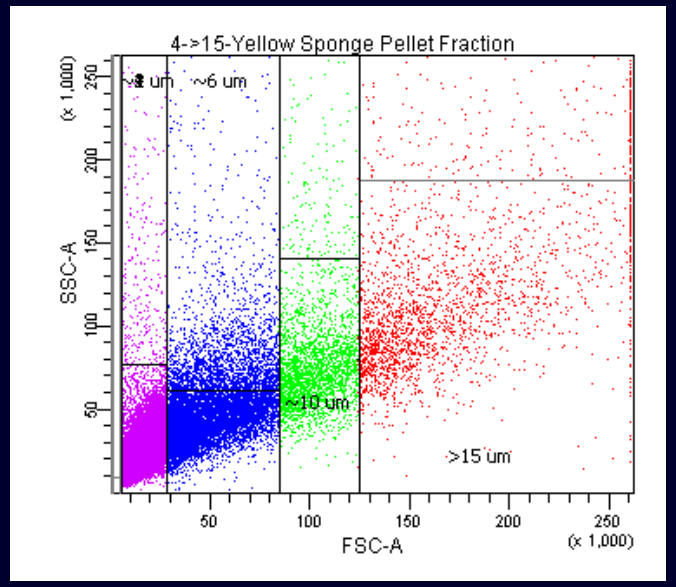


Sponge cells | **"Entotheonella palauensis"** | **Unicellular cyanobacteria** | **Unicellular bacteria**

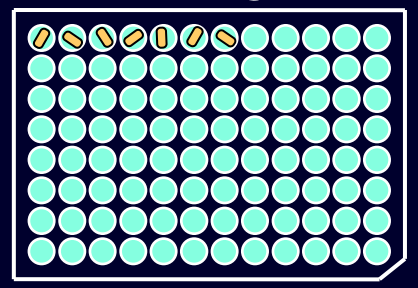
Courtesy of Joern Piel

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

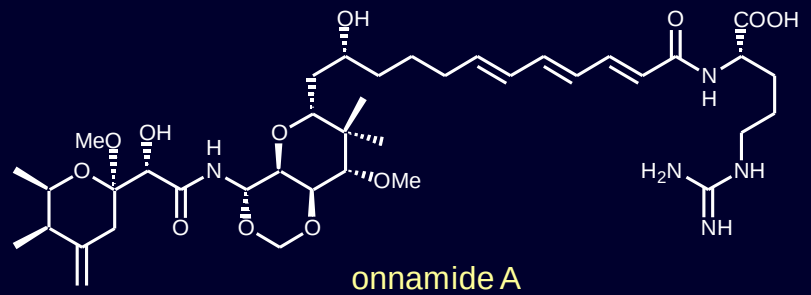
Localization of the Onnamide Genes by Single-Cell Analysis



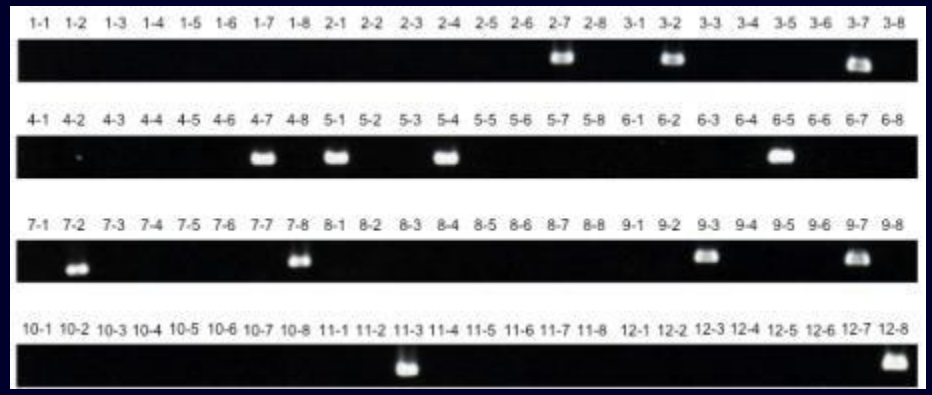
Fluorescence-activated cell sorting (FACS)



Microtiter plate with single cells



onnamide A

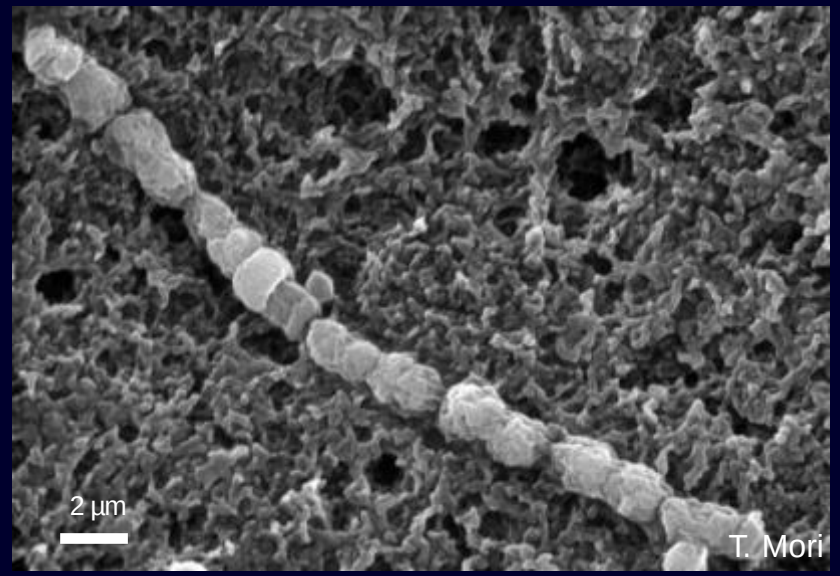


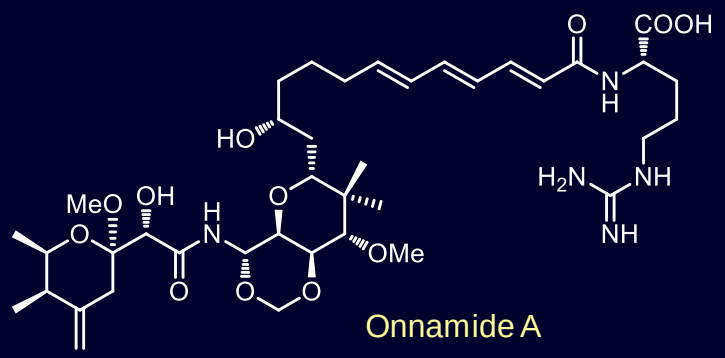
Several wells positive for onnamide genes

Courtesy of Joern Piel

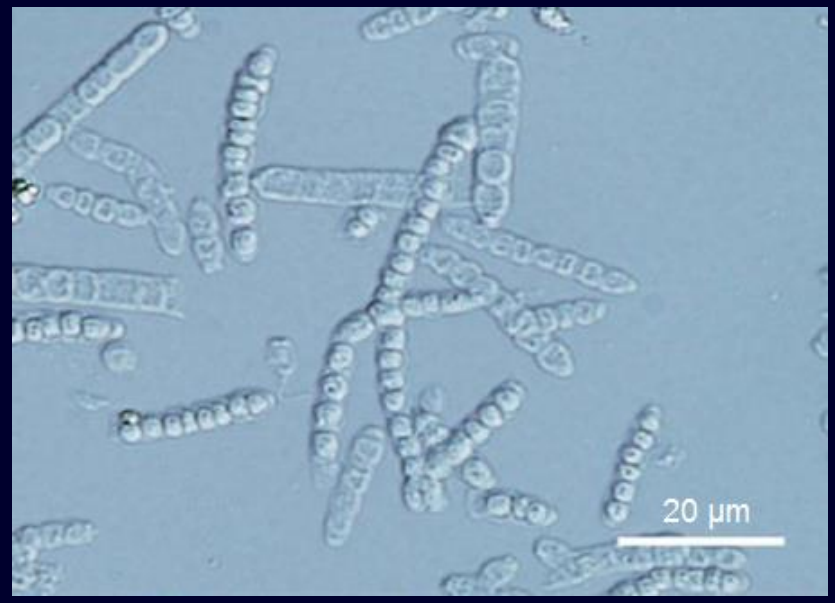
M. Wllson *et al.*, *Nature* 2014

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





Onnamide A



Courtesy of Joern Piel

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

ET743 and Close Relatives!

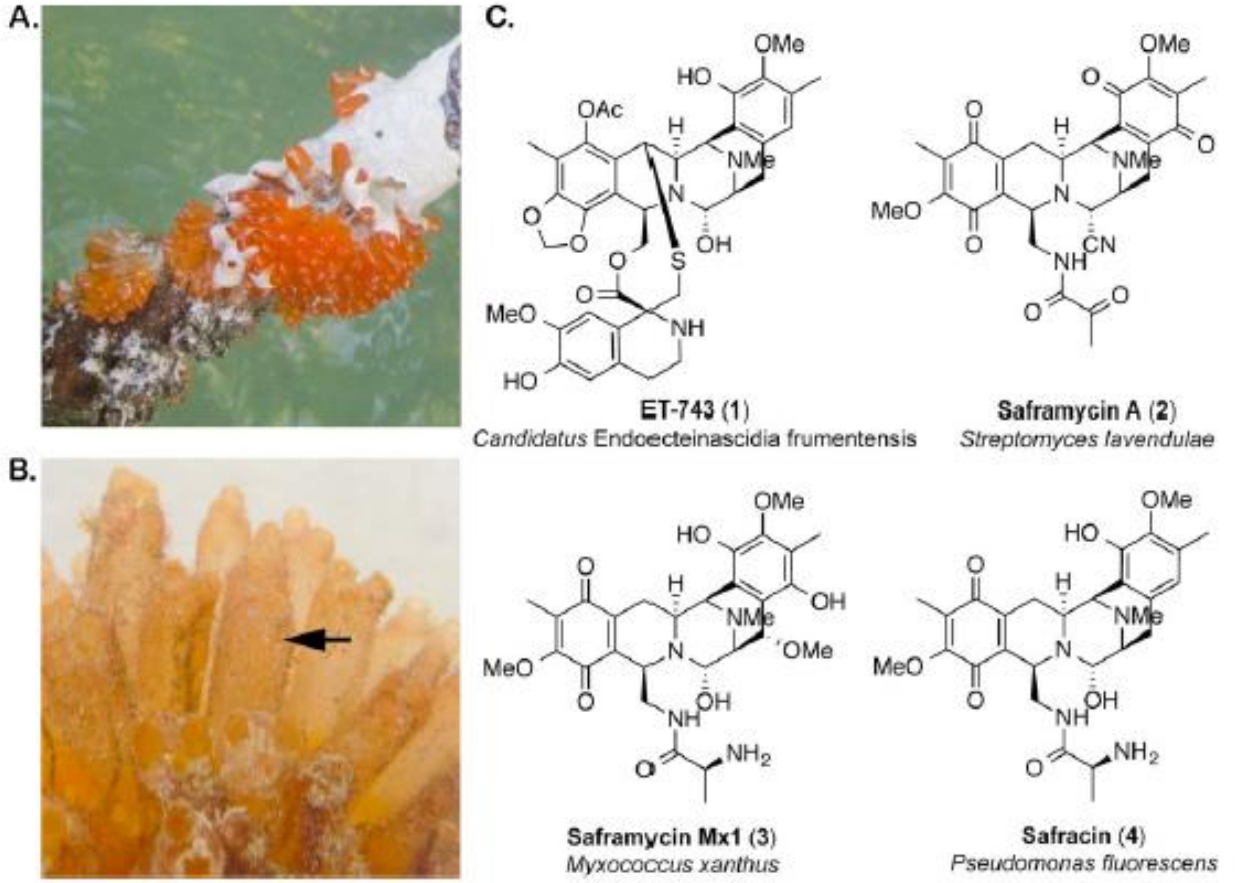


Fig. 1. A. Tunicate colonies growing on the root of a mangrove tree in the Florida Keys. B. A tunicate colony composed of individual zooids (indicated by arrow). In this study, we sequenced the metagenomic DNA from four zooids. C. The chemotherapeutic compound ET-743 (**1**) and three natural products from cultivable bacteria that share a similar tetrahydroisoquinoline core.

EU Approved 2007; USA 2015

Schofield et al, Env. Micro. 2015

Biosynthetic Pathway of ET743 in Candidatus E. frumentensis

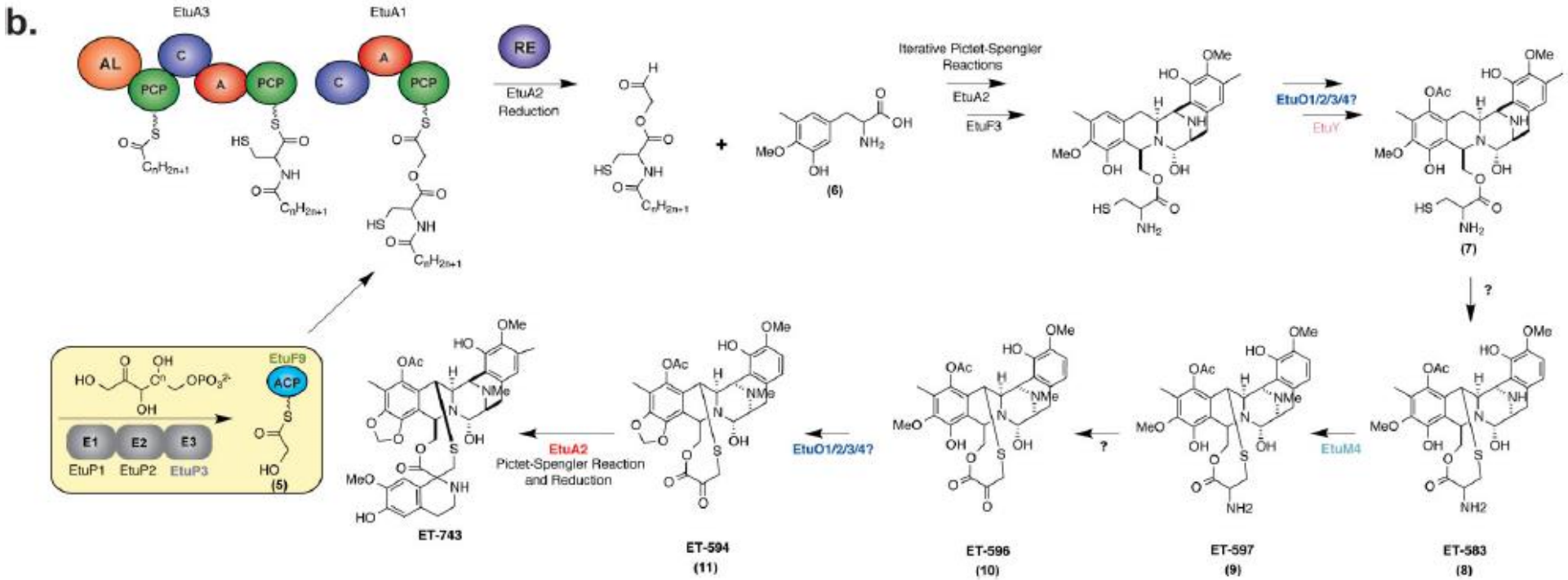
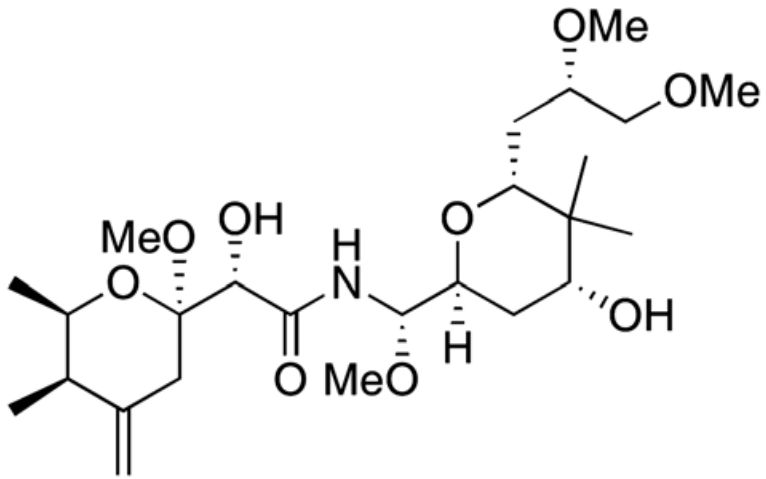


Fig. 4. The identification of new genes with suspected involvement in ET-743 biosynthesis. The genes and their putative roles are also depicted in Table S5.
A. New ET-743 biosynthetic genes were identified upstream and downstream of the original ET-743 biosynthetic gene cluster (outlined in black). Gene products are classified according to the corresponding colour key.
B. A condensed ET-743 biosynthetic pathway illustrating proposed new steps based on analysis of the complete genome. Coloured steps represent new enzymes or new roles for previously identified enzymes. An updated proposal for the complete biosynthesis of ET-743 is depicted in Fig. S6.

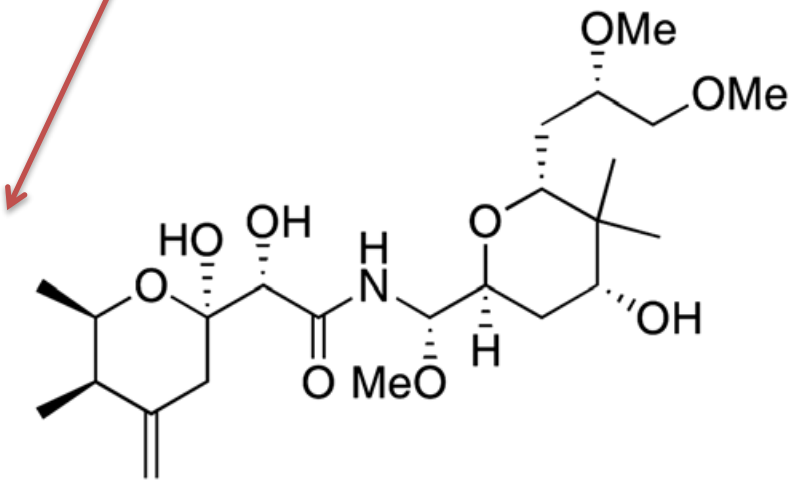
Schofield et al, Env. Micro. 2015

A Brazilian Connection I
(Paederus beetles that can "scuba dive!!")

Plus



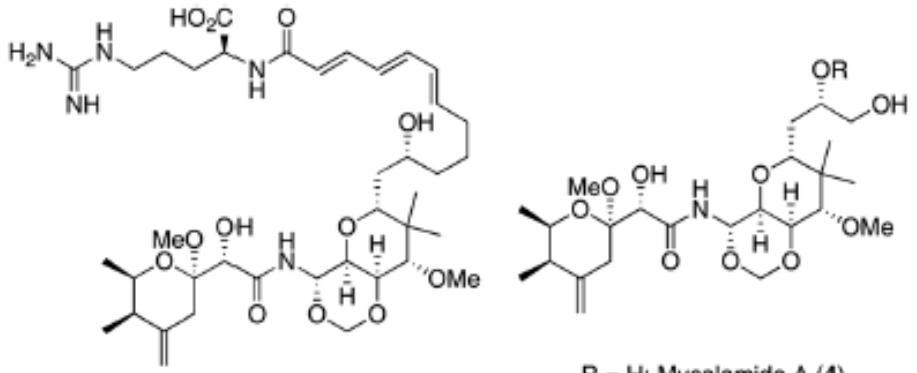
Pederin (**1**)



Pseudopederin (**2**)

Mosey & Floreancig, NPR 2012

Mycalamides and Similar Molecules



Onnamide A (**3**)

R = H: Mycalamide A (**4**)
R = Me: Mycalamide B (**5**)

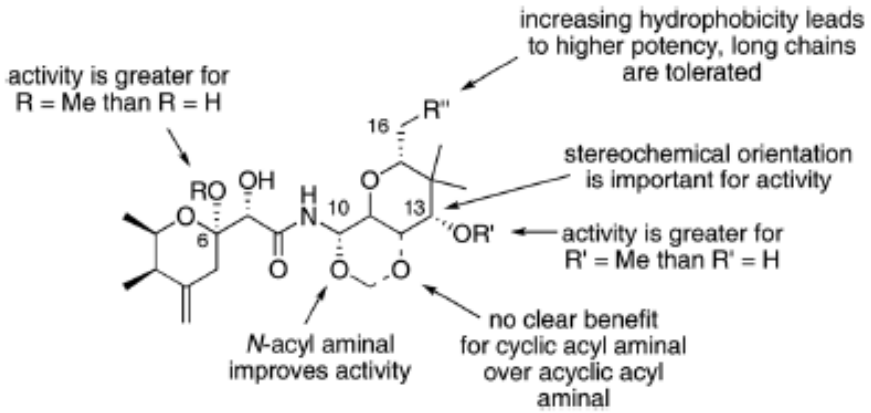
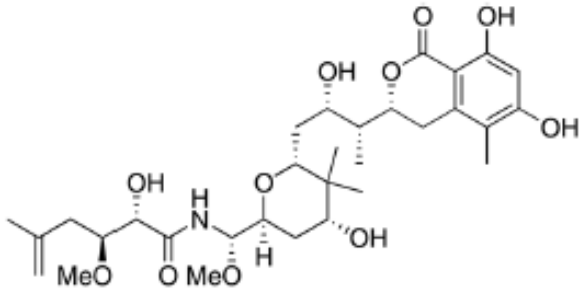
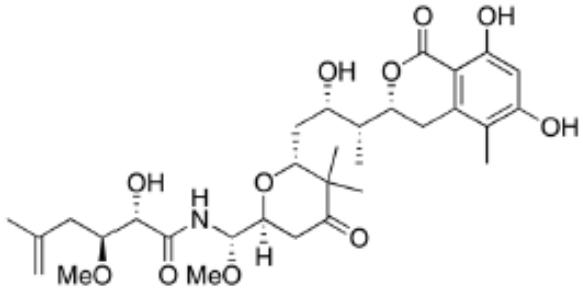


Fig. 7 Natural product-derived structure–activity relationship for the pederin family of molecules.



Psymberin/Irciniastatin A (**32**)



Irciniastatin B (**33**)

Mosey & Floreancig, NPR 2012

SAR from Synthesis of Pederin "Look-a-likes"

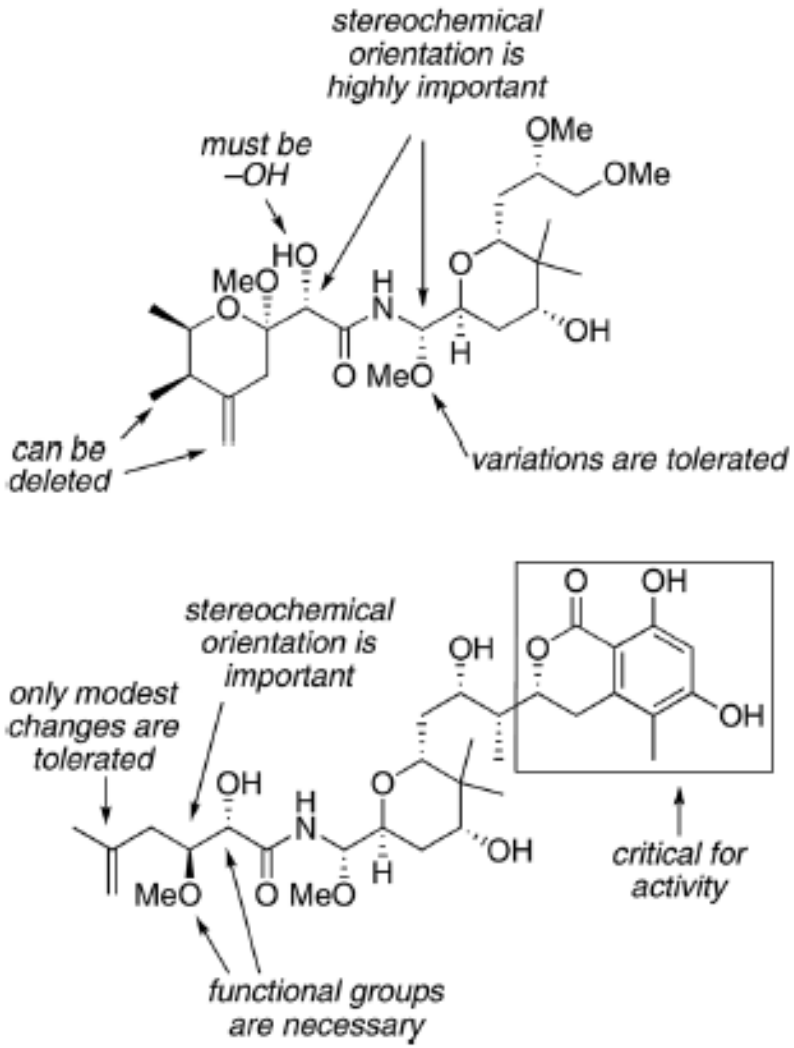


Fig. 15 Synthesis-derived structure–activity relationships.

Mosey & Floreancig, NPR 2012

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.

If we now add to this "tour-de-force", the paper from Sherman's group above, then add the work from Hong Kong & Scripps Oceanographic on didemnin B from free-living microbes, then I think that it can be safely said that this is possibly the case for the majority of marine-nvertebrate-derived secondary metabolites.

Finally Even Fungi have Endophytes !

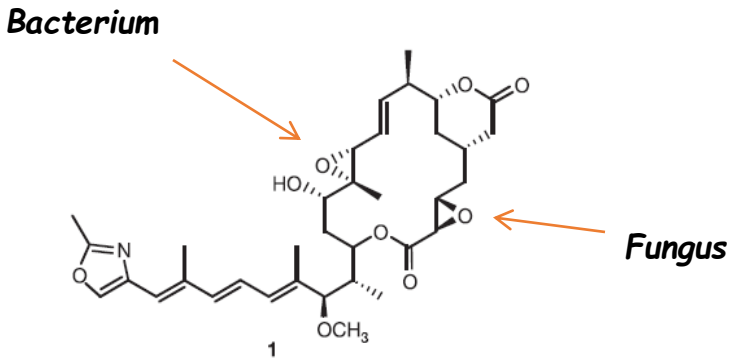
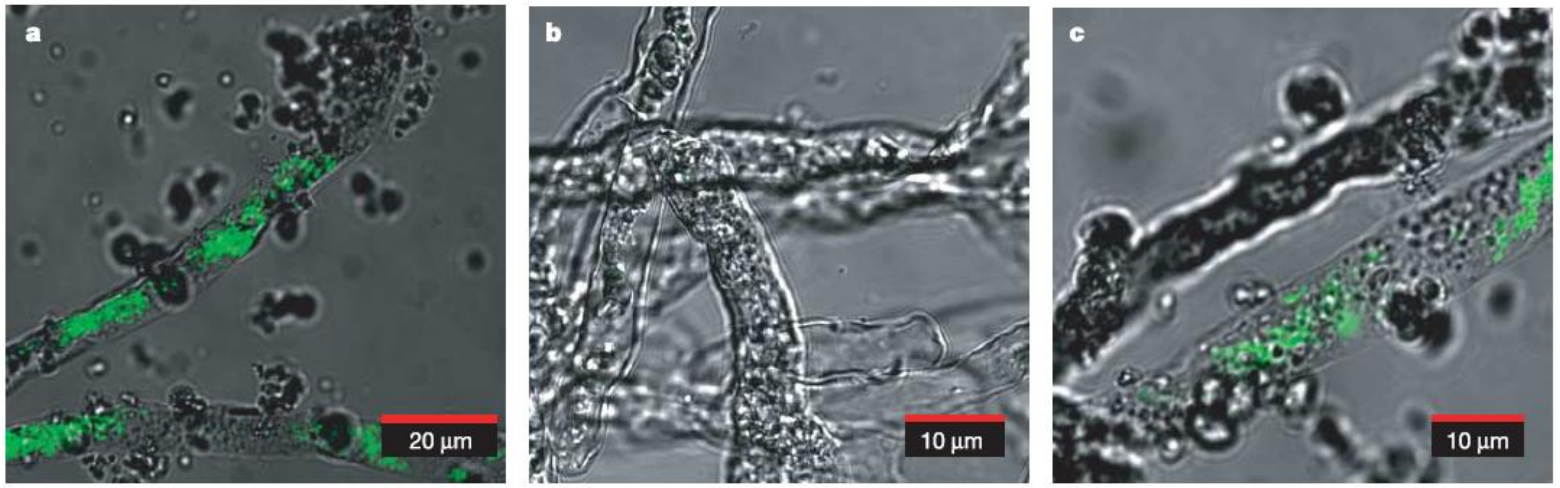


Figure 1 | Structure of rhizoxin, the causal agent of rice seedling blight, isolated from *Rhizopus* sp.

Hertwick from Jena (Nature 2005) reported (and proved) that Rhizoxin was produced by an endophytic Burkholderia.

Subsequently he has isolated and cloned the biosynthetic pathway.

Revised in 2012; now a tripartite system with mono-epoxy precursor being given second epoxy group by the fungus.



Lackner et al, AEM, 2009, 75(9), 2982-6

Many Thanks for Your Attention