Endophytes

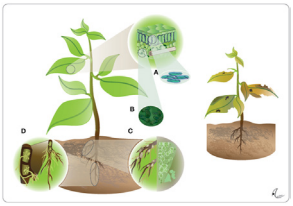
Introduced by De Bary in 1866. Afterwards, Bacon and White define them as "microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effect."

Endophytes have been found in every plant studied to date.

The most studied fungal endophytes belong to the ascomycetous family Clavicipitaceae.

Source of bioactive natural products: many of them occupying millions of unique biological niches (higher plants) growing in so many unusual environments.

Reservoir of unique chemical structures involved in host plant protection and communication mechanisms.



The plant as the extended phenotype of endophytes. Endophytes including fungal leaf endophytes (A), bacterial leaf endophytes (B), Rhizobia (C), and arbuscular mycorrhizal fungi (D), can infect all organs of a plant, which in their presence exhibits what we usually know as its normal phenotype.

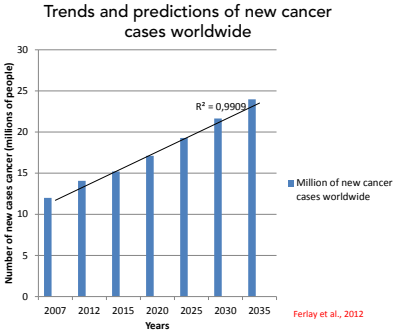
Partida-Martinez Laila P. Pamela, Heil Martin. 2011. *The microbe-free plant: fact or artefact? Frontiers in Plant Science*. Volume 2.

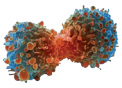
Cancer illness

Chronic illness, which occurs when alterations of genetic material create an abnormal function leading to unregulated proliferation of cells in the body.

Remains as one of the most predominant illnesses causing death: more than 10 million people are identified each year worldwide.

Environmental stresses such as infection, food additives, chemicals and air particles could create damages in the cells: DNA alteration is a serious consequence leading to carcinogenesis.





Lung cancer cell during cell division - taken with a scanning electron microscope. http://www.research.bayer.de/de/krebszellen-im-visier.aspx

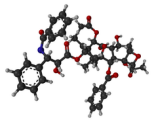
Main goals

1. Studying and summing up the development of clinic mycology focused on the findings of anticancer compounds synthesise by endophytes.
2. Studying the most important new compounds found until now.
3. Studying methods to produce endophytes' second metabolites in vitro: biotechnology and new challenges.

ENDOPHYTES AS PRODUCERS OF ANTICANCER COMPOUNDS

Paclitaxel alkaloid

Fungi: Taxomyces andreanae / Host plant: Taxus brevifolia
Against: Breast, ovarian, lung, head and neck cancers
Action mechanism: Bind to polymerized tubulin promoting microtubule formation and microtubule stabilization against disassembly inhibiting mitosis and therefore cancer growth.
Other sources: Many other endophytic fungi (residing in plants of the Taxus genus or other: Phomopsis sp.)
Production: Taxol®, Onxal®; semi-synthesis of the anti-cancer drug starting from the precursor desacetylbaccatin III (isolated in sufficient amounts from needles of Taxus sp.)



3D structure of Paclitaxel http://ca.wikipedia.org/

Colony of DSE fungus Phialocephala fortinii Wang et Wilcox in Petri dish. M.Vohník, http://www.ibot.cas.cz/mykosym/mycorrhiza.html

Podophyllotoxin lignan

Fungi-Host plant:
Trametes hirsuta - Podophyllum hexandrum / Phialocephala fortinii - P. peltatum
Fusarium oxysporum- Juniperus recurva
Aspergillus fumigatus - J. Communis
Against: Hodgkin's disease, large cell lymphomas, pediatric leukemia, testicular tumors, and lung small cell carcinoma
Action mechanism: Inhibition of topoisomerase II blocking the ligation step of the cell cycle harming the integrity of the genome apoptosis and cell death
Production: Etoposide (Toposar®, VePesid®, Etopophos®) and Teniposide (Vumon®)

Vincristine and vinblastine alkaloids

Fungi: Fusarium oxysporum
Host Plant: Catharanthus roseus or Vinca rosea
Against: Lymphoma and leukemia
Action mechanism: Arrest mitosis by binding to tubulin dimers which inhibits their assembly to microtubule structures
Production: 500 kg of leaves 1 g of purified vincristine (from $1 to $3.5 million/kg) research in alternative sources needed

Catharanthus roseus http://www.guide-to-houseplants.com/periwinkle-flower.html

Leave, fruit and tree of Camptotheca acuminata http://www.tasmanianarboretum.org.au/plant_list.shtml

Camptothecin (quinoline alkaloid)

Fungi: Fomitopsis sp., Alternaria alternata, and Phomposis sp.
Host Plant: plant species from Asterid clade. Originally obtained from Camptotheca acuminata (Cornaceae) but occurs also in Apocynaceae , Rubiaceae, or Gelsemiaceae or Icacinaceae.
Against: human ovarian, small lung and refractory ovarian cancers. Methanolic and ethyl acetate extracts against colon and breast cancer cells
Action mechanism: CPT binds to the topoisomerase I (which catalyze and guide the unknotting of DNA during DNA transcription) and DNA complex resulting in a ternary and stable complex. Prevention of DNA re-ligation (DNA damage) apoptosis of cancerigen cells
Production: Two CPT analogues used in cancer chemotherapy today: topotecan and irinotecan. New nanomedicine drug: CRLX101

Sclerotiorin

Endophyte-Host plant:
Cephalotheca faveolata - Eugenia jambolana
Against: colon cancer cells (HCT-116)

Ergoflavin

PM0651480- Mimosops elengi (Sapotaceae).
Against: renal, lung, human pancreatic, human colon and non small cell lung cancer.

Tauranin

Phyllosticta spinarum - Platycladus orientalis (Cupressaceae)
Against: non small cell lung, breast, metastatic prostate and pancreatic cancer, central nervous system glioma

Phomopsin A

Phomopsis sp - bark of an unidentified Mangrove plant collected in China
Against: human nasopharyngeal epidermal carcinoma and human multidrug resistant cells

Trichodermamide C

Eupenicillium sp. - Glochidion ferdinandi (Euphorbiaceae)
Against: human colorectal and human lung carcinoma

Cajanol isoflavone

Hypocrea lixii - Cajanus cajan (Pigeon pea roots)
Against: Human prostatic adenocarcinoma, human breast cancer, human lung carcinoma cells

DISCUSION

Levels of chemicals in our environment are increasing day after day (pesticides, heavy metals, dioxins, PCB's ...) have been related to a huge amount of cancer lines. (Danjou., et al 2015, Jarup 2015)

Cancer is an emerging disease . In 2012 8,2 million people died as a result of cancer and 14 million new cases emerged new effective methods and medicines need to be discovered.

Endophytes: During long coexistence process with their hosts develop many significant and novel characteristics adapted themselves to their special microenvironments by genetic variation (uptake of some host DNA into their own genomes) ability to biosynthesize some phytochemicals originally produced by the host plant [Tan and Zou, 2001].

Challenges:
Finding new metabolites synthesized by endophytic fungi with the possibility of growing in 'in vitro' conditions and being exploitable.
Discovering the mechanisms of horizontal gene transfer between host and endophyte (or vice versa)
Research in alternative sources and methods, without damaging the host plant or the respective ecosystem.
In spite of all the studies done, few compounds such as paclitaxel (taxol®), camptothecin, vincristine (Oncovin®), vinblastine (Velban®) and podophyllotoxin (etoposide and teniposide) have been industrialized as anticancer drugs.
Research community should focus their effort on molecular studies and optimization of fermentation conditions to scale up the production of interesting metabolites.

BIOTECHNOLOGY

- Alternative method to produce natural metabolites manipulating the parameters influencing the growth and metabolism of cultured tissues
Recent gene sequencing studies of fungal secondary metabolism the number of expected natural products.
- Difficulties: Certain biogenetic fungal gene clusters are apparently not expressed under the usual laboratory culture
- Factors which influence the production of secondary constituents by endophytes (difficult or impossible to control):
- Developmental stages of the fungal culture, vegetative growth and sexual/asexual development , regulation of the nuclear protein LaeA...
- General environmental factors and specific needs: carbon and nitrogen sources, temperature, light, and pH, host plant interaction and communication, competition among endophytic fungi and bacteria.
- Now-a-days production:
Vinblastine and vincristine still obtained from cultivated Catharanthus roseus.
Paclitaxel from Taxus brevifolia and Podophyllotoxin from Podophyllum sp. semi-synthetically produced from natural metabolites isolated from in vivo sources (biotechnology).

Development of a plant cell suspension culture: Sterile explants from the whole plant (a) are plated on solid culture medium. With the correct nutrients and hormones combination, explants grow into a callus of dedifferentiated cells (b). Callus cells are transplanted into liquid media, creating a suspension culture (c), which can be scaled up for growth and production in a controlled bioreactor (d). Wilson, S. and Roberts., S.C. Recent advances towards development and commercialization of plant cell culture processes for the synthesis of biomolecules. (2012) Plant Biotechnology Journal

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