Screening of Plant Cell Cultures for New Industrially Interesting Compounds

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Introduction

- Plants: sources of food, beverages, fibers, building materials, poisons, drugs, colorants, flavors, fragrances and insecticides
  - Many natural products are now produced as specialty chemicals for applications in the food, pharmaceutical, flavor and fragrance industries.
- At present, a total of 119 chemical substances used as drugs are extracted from plants.
- So far, only small percentage(5~10%) of all plants has been screened for useful compounds with the aid of modern scientific tools.
  - Sleeping Giant for drug development!
- What are the advantages of screening natural products for biological activities when contrasted with synthesizing new drugs?
  - Highly complex structures (e.g. chiral centers) which are difficult to synthesize with purely chemical means
  - Understanding the relationship of plant-insect, plant-microorganism and plant-plant, secondary metabolites are considered to serve a certain function for the organism in which they are produced.

- Synthesized chemical (SC) .vs. Natural compound (NC)
  - SC: directed at certain compounds (e.g. based on molecular modeling studies), which will lead only lead to improved drugs for already known receptors
  - NC: much more probable to show totally new modes of action in broad screens

- How can we best exploit the potential of plants?
  - To perform detailed studies of plants hat have history of being used for a particular purpose, e.g. a medicinal plant
    - Plant material is difficult to obtain, thus limiting the possibilities for further development for industrial production. (e.g. Taxol)
    - So, plant cell cultures are an interesting option for production.
      - *However, this means of production is **NOT** feasible for all compounds. For many natural products, it has been impossible to obtain higher production in cell cultures than in plants and furthermore not at all. (e.g. vinblastine)*
    - If interesting compounds are obtained by plant cell culture, one is not limited by insufficient material.
    - From the point of view of patent protection, production by means of plant cell cultures is interest. (especially, in rare plants)
  - To randomly screen plants for certain biological activities or other traits on interest

Here we further discuss the screening of plant cell cultures for new pharmacologically interesting compounds!!

Screening Methods

- ①*in vivo* general screening, *in vivo* assay for specific activity and ②screening of microorganisms and viruses for antibiotic and antiviral activity on intact organisms
  - ①
  - Disadvantage: relatively large amounts of extracts are needed.
  - Advantage: completely new drugs with yet unknown modes of action can be found.
  - Only structure of newly founded compound might be new and the behavior toward other receptors might be different. (i.e. resulting in a compound with possibly different or reduced side effects)
  - ②
  - Not require large amounts of extracts
  - Chances of finding antimicrobial activities must be regarded as high, since plants defend themselves against microorganisms by producing secondary metabolites with antibiotic activity.

- *in vitro* general screening and *in vitro* assay for specific activity using isolated organs
  - The use of isolated organs is decreasing because of the wish to reduce the number of animal experiments.

- Screening using isolated cells for specific activities (i.e. human, animal and plant cells)
  - The use of isolated cells is increasing (intermediate between the in vitro 'biochemical assays' and the whole animal)
  - Effective and widely applied in the screening of antitumor activities

- ①[Receptor-binding assays, enzyme assays], ②[chemical screening and immunochemical screening at the molecular level]

  ①
  - Widely used to test new compounds for interesting known biological activities
  - Very sensitive and specific
  - Enabling them to be performed with small amounts of crude extracts
  - Quite rapidly and full automatization is possible
  - Can be applied subsequently to a test system with a higher level of complexity, i.e. isolated cells or whole animals

  ②
  - Particularly aimed at finding new sources for known compounds

Extracting Plant Materials

- Factors to be considered
  - Ease of the method, the risk of missing active compounds, the chance of finding active compounds and the problems of testing certain types of extracts during screening
- In plants, a large number of compounds is present, encompassing a broad spectrum of polarities. → no single solvent is able to extract all compounds in a single step. → **inherent to screening**
- Without the effective extraction method, the subsequent test system will give the wrong information to us.
- Dichloromethane-methanol(1:1) as a general applicable solvent for the screening of plants for antitumor activity. (to dissolve 80% of all soluble plant secondary metabolites)
  - Tested several solvents for the extraction of plant cell culture: water, alcohol, chloroform, toluene and MC-MEOH(1:1)
  - Water extracts: sugars & amino acids
  - Ethanol: sugars, amino acids and fats/fatty acids
  - Chloroform & Toluene: fat/fatty acids
- A problem encountered in using apolar solvents was **how to dissolve the extracts in water**, because most test systems require aqueous solutions.

Results of Screening Programs of Plant Cell Cultures

- At least three pharmaceutical industries (e.g. Nattermann in Germany) ran screening programs fro new pharmacological lead compounds from plant cell cultures.

- Screening Program of the Nattermann Company
  - Selection of plants(100%)
    - Not investigated, no medicinal plants
    - small and/or rare plants
    - Preferably endemic or legally protected
  - Callus initiation(50%)
    - Series of media is tested initially
    - Reduction to two media with growth
    - 6 to 8 passages till stabilization
  - Biological screening(5%)
    - 50 to 100 callus gives 20 to 50 mg extract
    - *In vitro* (receptor-binding assay) or *in vivo* testing
    - Selection of interesting cultures
  - Suspension culture (79 upscalings)
    - Initiation on liquid medium (same as callus)
    - 10 to 12 passages till stabilization
    - Scale-up in 30-l fermenters
  - Isolation of active compounds (150 components)
    - Freeze drying of cells
    - Crude fractionation on XAD
    - Fractionation on reversed phase
    - Preparative HPLC
  - Identification of active compounds
    - 40 active compounds, 26 anti-flammatory of 7 are new structures

- Some results of this screening program have been poublished and patented. (e.g.1 isoquinoline alkaloid jatrorrhizine and two isomeric dehydro diconiferyl alcohol glucosides from *Plagiorhegma dubium* Maxim; e.g.2 podoverin A and podoverin B from *Podophyllium versipelle* callus culture; percine and pericalline from *Picralima nitida* callus culture)

- Generally the plants had higher activity and for all cell cultures in which activity was found, the plants also exhibited activity. The opposite, however, did not hold true. In other words, plants are a better source for finding activity.

- Biological activity was most pronounced in species known to contain toxic secondary metabolites or used in traditional medicine. On the other hand, common agriculture plants demonstrated little or no activities.

- A remarkable finding in the screening was that in numerous receptor-binding assays a potentiation of the binding of the test substrate was found under the influence of the extracts. This potentiation of binding is probably due to nonspecific effects, i.e., no single compound seems to cause this effect.

- A general problem in the screening – many plants contain specific, common compounds that cause a positive response in certain receptor-binding assays. (e.g. gamma-aminobutryic acid (GABA), glutamic acid, trytamine and adenine) For large scale project, it would be necessary to develop tools to eliminate the false-positive results at an early stage.

- A chemical determination (by GC or HPLC or ^{1}HNMR) of the compounds that might interfere in the various assays should be of great help. However, selective extraction methods avoiding these false positives would be very difficult to develop.

- Although screening at the level of calli has the advantage od reducing the number of cultures of interest, it must be kept in mind that often the step from callus to suspension culture results in a considerable reduction in the levels of certain secondary metabolites. Sometimes a complete loss of productivity for certain products may occur.

- **Plant cell cultures are an excellent source of new compounds.**

Conclusions

- Screening of plant cell cultures for new biologically active compounds is **feasible.**
- **In general, more activities will be found in extracts of plants than in extracts of cell cultures.**
- **The use of plant cell cultures is of particular interest in case of rare plants.**
- The chances of finding interesting activities are increased by using **plants known to be toxic or used in traditional medicine.**
- The major advantage of using cell cultures is **the direct availability of production method.**