Integrated analytical approaches towards marine natural products discovery

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SUBMARINER Blue Biotechnology Cooperation Event
Kiel, 10th May, 2012
Current discovery project activities

- **Center for Microbial Biotechnology**
  (2003-2012, FTP ca. 7 mill Euro’s)

- **Discovery of marine bioactive bacteria and natural products**
  (2008-12, DSF, ca. 2 mill Euro’s)

- **PharmSea** (EU FP7, 2012-2016, ca. X mill Euro’s)

- **HABFISH** (DSF, 2012-2016, ca. 3 mill Euro’s)
Intelligent versus high-throughput screening

• How can we run the smallest amounts of microbial or algal extracts in our bioassays and test as much chemical diversity as possible?

• How can we avoid redundancy in testing organisms producing the same profiles of natural products?

• How can we avoid redundant discovery of the “usual suspect bioactives”?

• Or at least identify them ASAP?
Our integrated discovery approach

Thousands of microbial strains => 100 thousands of compounds

1) Cultivation of microbes on few selected media
2) Micro-sample preparation, raw extracts
3) Direct metabolite fingerprinting (DiMS)
4) Cultivation using several conditions (OSMAC)
5) Natural product compound libraries (LC-UV-MS)
6) Bioassays
7) Isolation and structural elucidation

New drug hit

1-5 new promising candidates

Larsen et al., Nat. Prod. Rep., 2005, 6, 672-690
Minute sampling for initial screening

- Cut three plugs
- Add solvent
- Extract ultrasonically
- Re-extract the plugs
- Transfer extract to clean vial
- Add solvent
- Redissolve ultrasonically
- Evaporate solvent
- LC compatible solvent
- Filtrate and analyze
Have we seen a similar mixture before?

Does the mixture contain single compounds we have seen before?

Does the mixture contain possible new bioactive compounds?
Chemoconsistency within fungal species!

Plug extracts from fungi cultivated at standard conditions analyzed by simple TLC
Direct Injection Mass Spectrometry - DIMS
Mass profiles are species characteristic!

A & B are *Aspergillus lentulus*

C & D are *Aspergillus fumigatus*
Works sometimes in marine bacteria

Vibrio coralliilyticus

- Seaweed s4053
- Sediment s2052
Maximization of chemodiversity

=> Only 1-2 representative strains selected from each chemotype (species)

Larsen et al., Nat. Prod. Rep., 2005, 22, 672
OSMAC – One Strain MAny Compounds

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Search in ACD database > 37180 compounds (source = alga)

3061 hits
Automated target analysis – Bruker MaXis

Advanced target analysis searching for 3000 compounds in 30 sec

Building on old and new data from own lab

Reference collection of ca. 1000 compounds
Functional group properties of natural products

Explorative Solid-Phase Extraction (E-SPE)

- **Strong anion-exchanger (SAX)**
  - Presence of carboxylic acids
- **Mixed-mode anion-exchanger (MAX)**
  - Presence of acids, incl. phenols
  - Relative polarity
- **Strong cation-exchanger (SCX)**
  - Presence of basic groups, such as amines
- **Size-exclusion (LH-20)**
  - Relative size of the compounds

E-SPE bioactivity profiling

*Pseudoalteromonas luteoviolaceae*

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- **Violacein**, known antibiotic
- **Indolmycin**, “new” antibiotic
UV-guided discovery of further similar hits

Source: 

Agilent Thought Leader Program

1.4 mill Euro donation for new UHPLC-QTOF and QQQ for Jens C. Frisvad!

“Agilent is increasing mind share and market share among leading academics of our best-in-class life science solutions and analytical instrumentation. With the Thought Leader Program, we select a few very influential thought leaders in well-defined focus areas and we contribute funds, products, and expertise to their research.”
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(PI, natural product chemistry)

and several other new partners ...

Agilent Technologies
Donation of new MS instruments