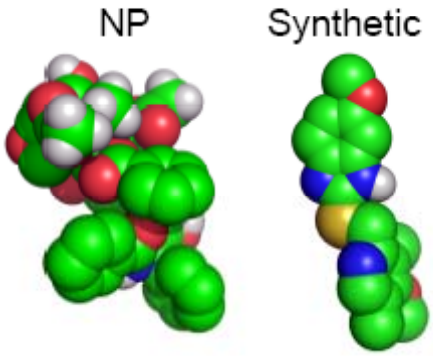
Actinomycetes secondary metabolites and drug discovery

Jean-Jacques Sanglier

questions

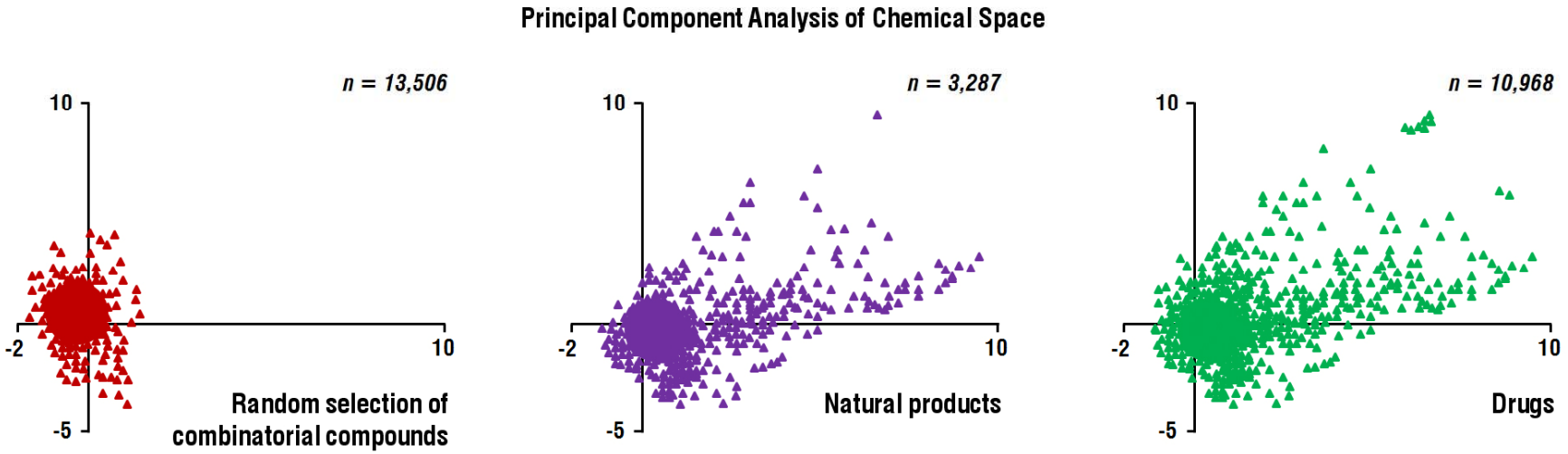
- Why natural products ?
- Why actinomycetes ?
- What is the present potential of actinomycetes?
- Which type of screening?
- conclusions

Why microbial natural products?



- Molecular complexity ⟶ activities
- Chemical diversity (chemical space)
- Selected over 3.5 BA of evolutionary pressure
- Affinity to cellular ligands
- Possibilities of engineering
- Discovery of cellular pathways and targets.

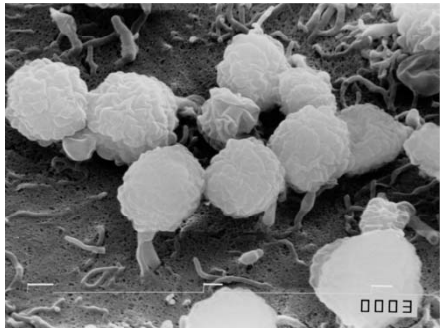
Chemical space

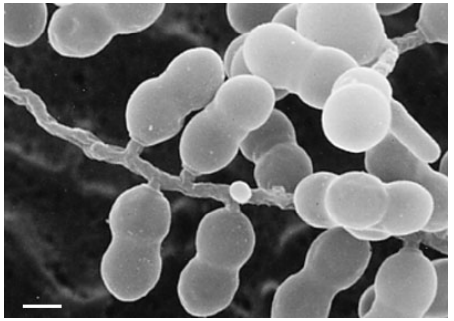


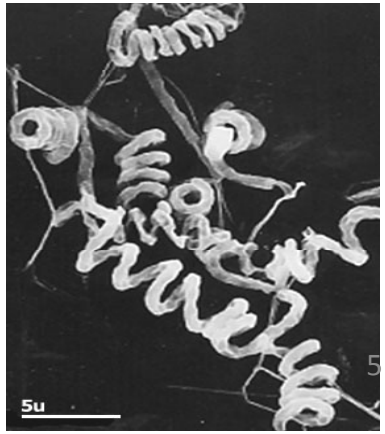
Vijramaditya G. Yadau, 2011)

Actinomycetes : definition

- Strains of Actinomycetes are Gram-positive, mycelium-forming (more or less!) prokaryotes with a high G+C content (70 to 74%) in their DNA.
- They display a more or less complex life cycle which involves the production of a large number of secondary metabolites.
- They have a quite long genome: 8-9 millions bases for *Streptomyces* (only 3.5-4.5 kb for *Mycobacterium*,…) and usually various clusters for secondary metabolites. Genetic lability is often observed.







Primary and secondary metabolism

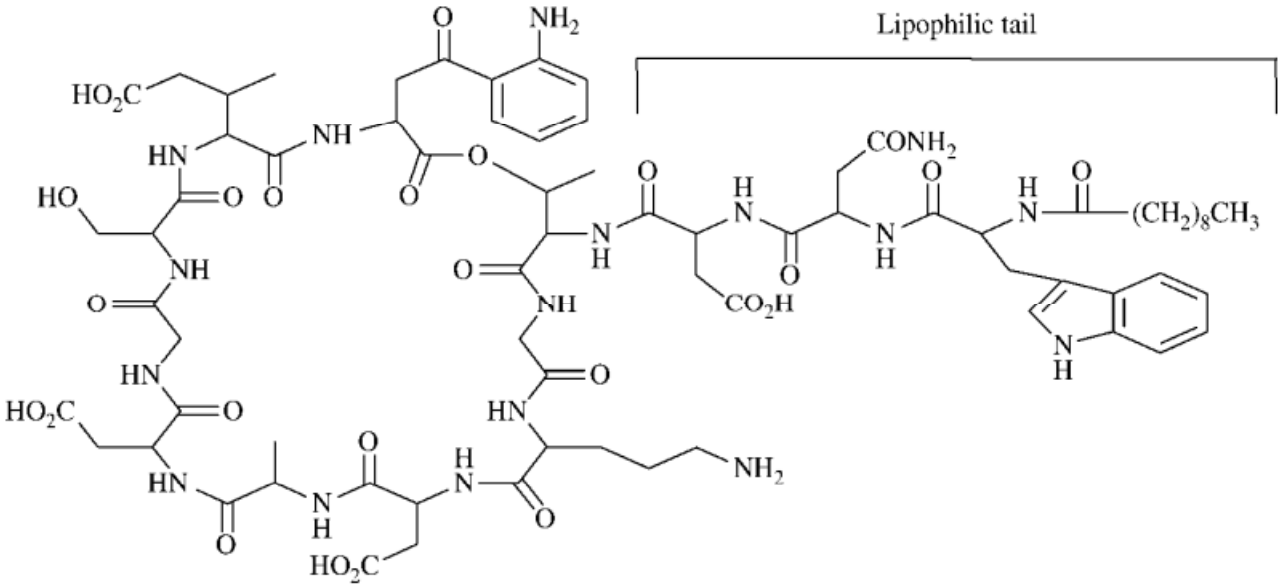
Over 10.000secondary metabolites from Actinomycetes described

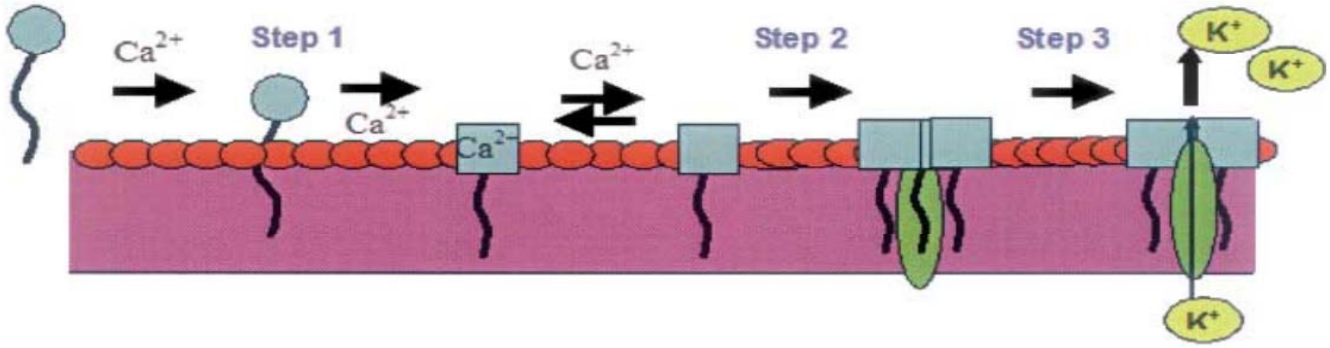
primary	secondary	examples
sugars	aminoglycosides	gentamycin
Amino acids and derivatives	Peptides B-lactams Alkaloids,…	thiostrepton, actinomycin, clavulanic acid, staurosporin,
Acetate, propionate,…	polyketides	Tetracyclin, erythromycin
mevalonate	terpenoids	
nucleosides	Nucleoside derivatives	nikkomycins

and hybrid molecules bleomycin, sanglifehrin

Special chemical bonds
Incorporation inorganic

daptomycin

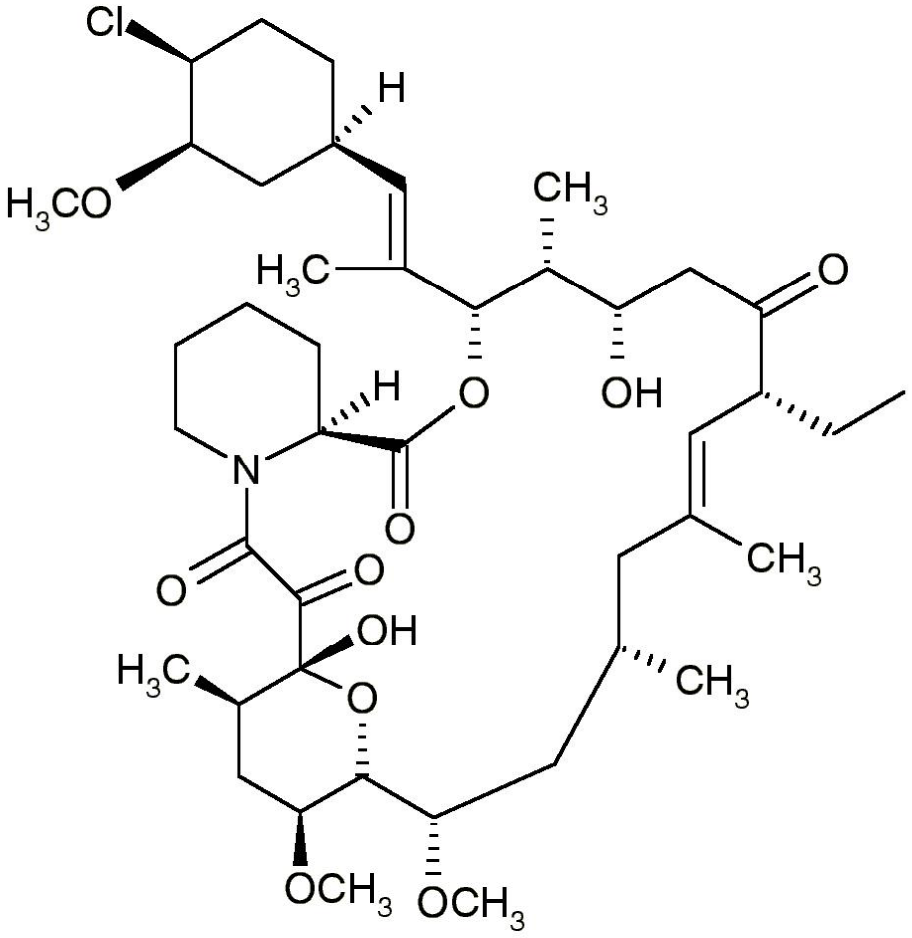




Binding to the cytoplasmic membrane, disruption, release ions, death

J. Antimicrob.Chemotherapy 2005 55: 283

Pimecrolimus (ascomycin)

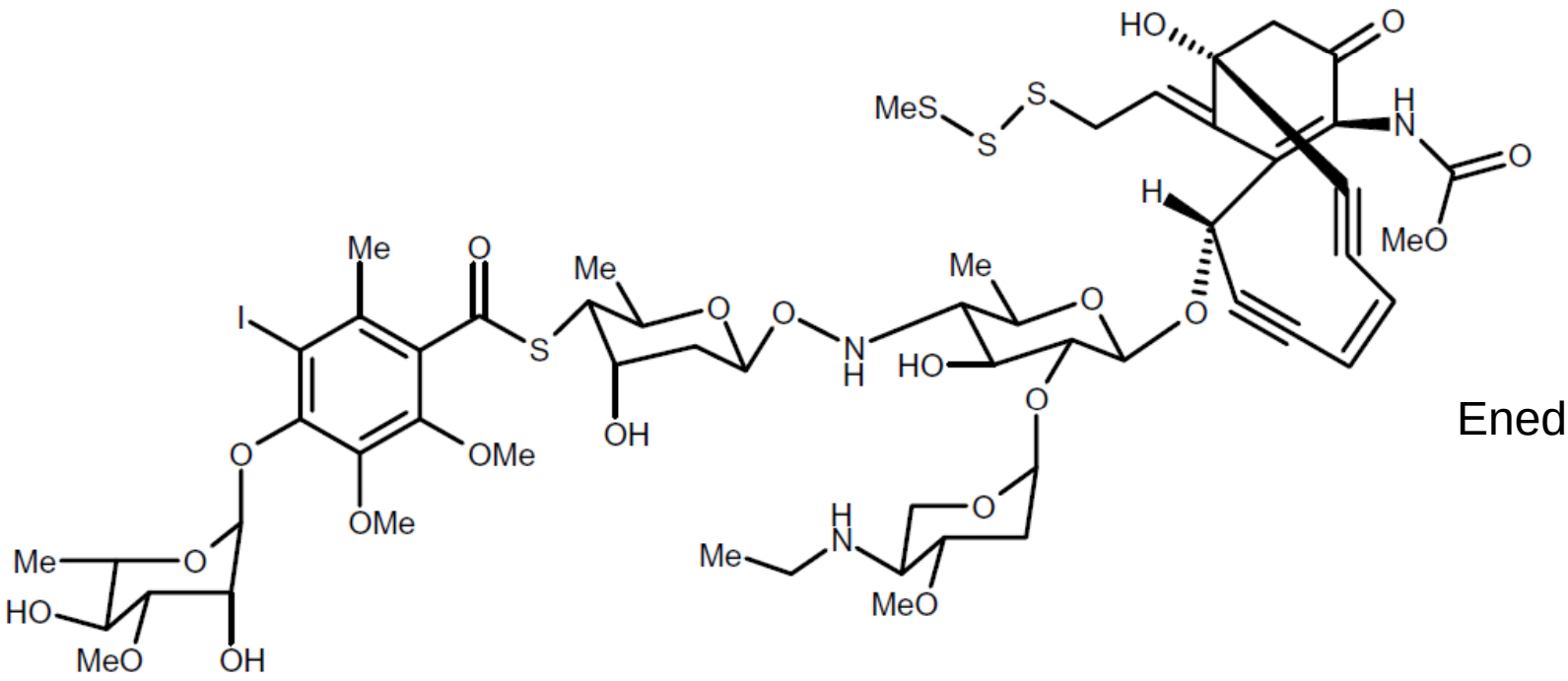


treatment of inflammatory skin diseases

blockage of T cell activation

J Eur Acad Dermatol Venereol. 2003,17:493

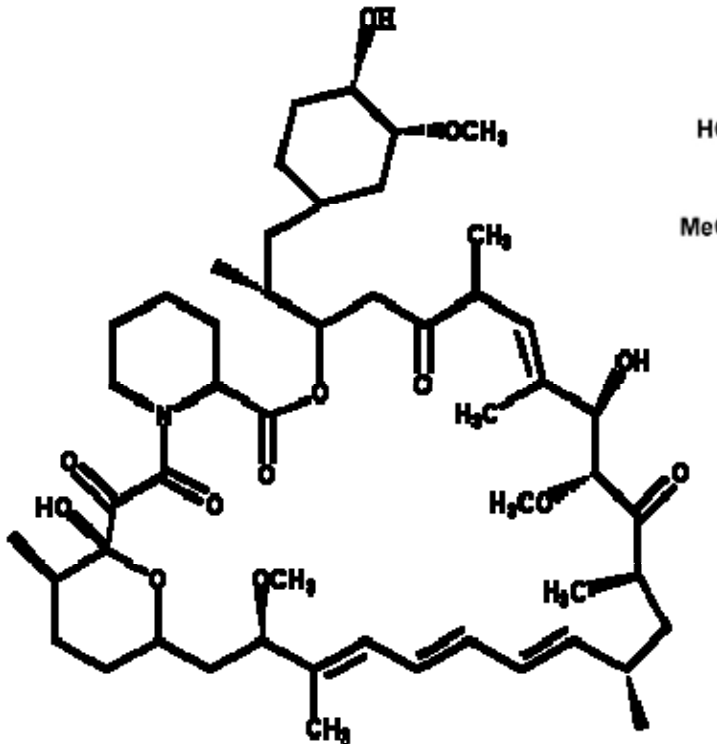
Calcheamycin



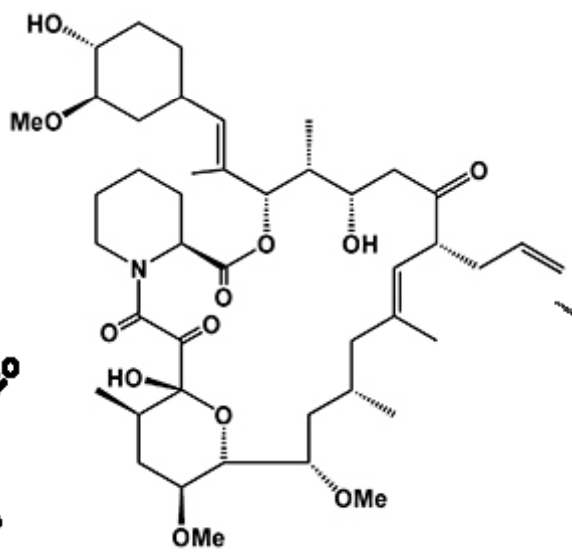
Enediynes

Binding DNA, breakage

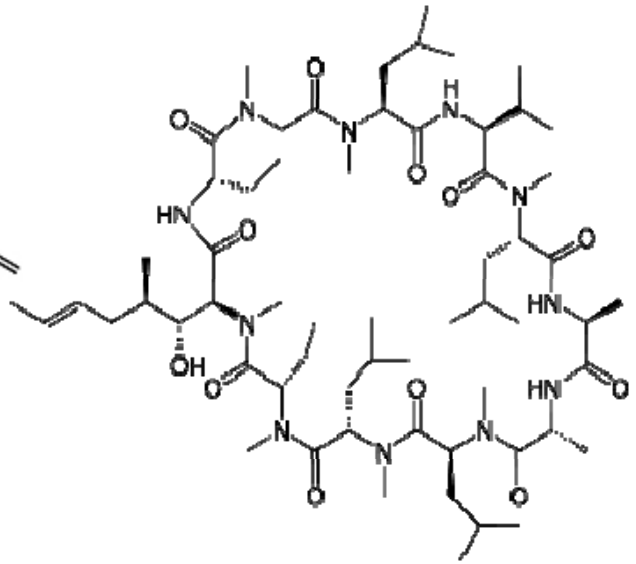
Natural immunosuppressants



rapamycin



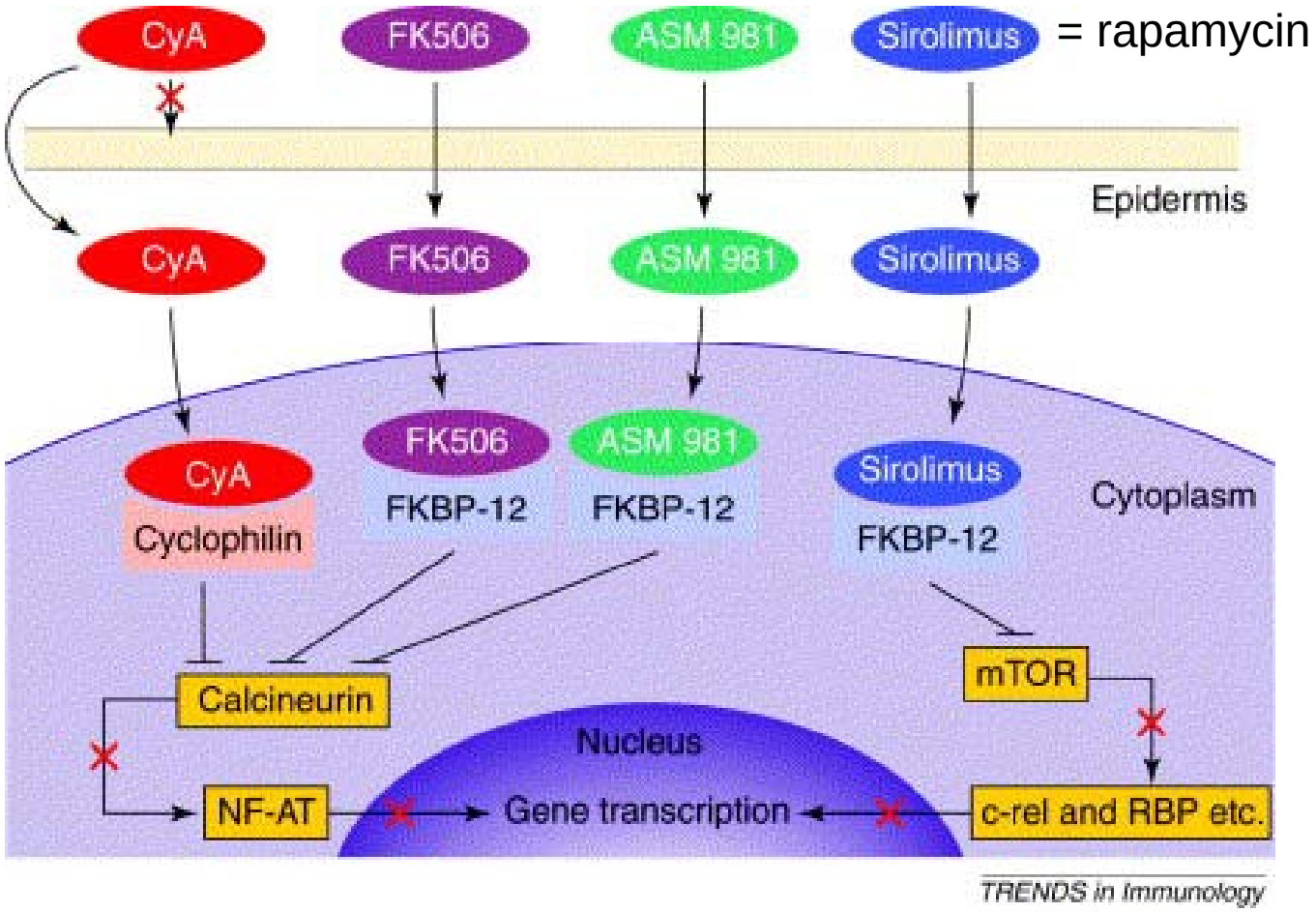
FK-506



cyclosporin

NP : pathfinders

mode of action of immunosuppressants



Why are actinomycetes excellent source of natural products ?

- various ecological types
- long chromosom with many biosynthetic gene clusters
- quite a good separation core region and accessory regions. A part with linerar chromosomes.
- main compounds synthetised on modular systems
- hybrid-molecules
- low specificity of various enzymes (e.g. actinomycins)
- post-tayloring (e.g. methylation, glycoloysation)
- capacity to build unique structures up (e.g. enediynes) and incorporate « minerals »
- importance of deletions, duplications, recombination, …..

Chromosome of Actinomycetes:

Case of *Streptomyces* (8.6 to 11.9 kb)

linearity

Core region : primary metabolism (33 to 45%)

Outer arms: most of secondary metabolism / differentiation
Variable = accessory genome

Deletions, duplications, transfert…..

Attention : not all Actinomycetes have a linear chromosome!

BMC Bioinformatics 2012, 13(Suppl 10):S8

Core genome components of representative *Streptomyces*

strains	genome size (Mbp)	Genes	% core in genome
S. avermitilis MA-4680	9.1	7765	41.9
S. coelicolor BCW-1	9.1	8300	39.4
S. scabiei 87-22	10.1	8901	36.5

Remarks:
-18-25 % core genome for secondary metabolism
- 23 – 37 secondary metabolism clusters per strain

Multiple gene clusters for secondary metabolism

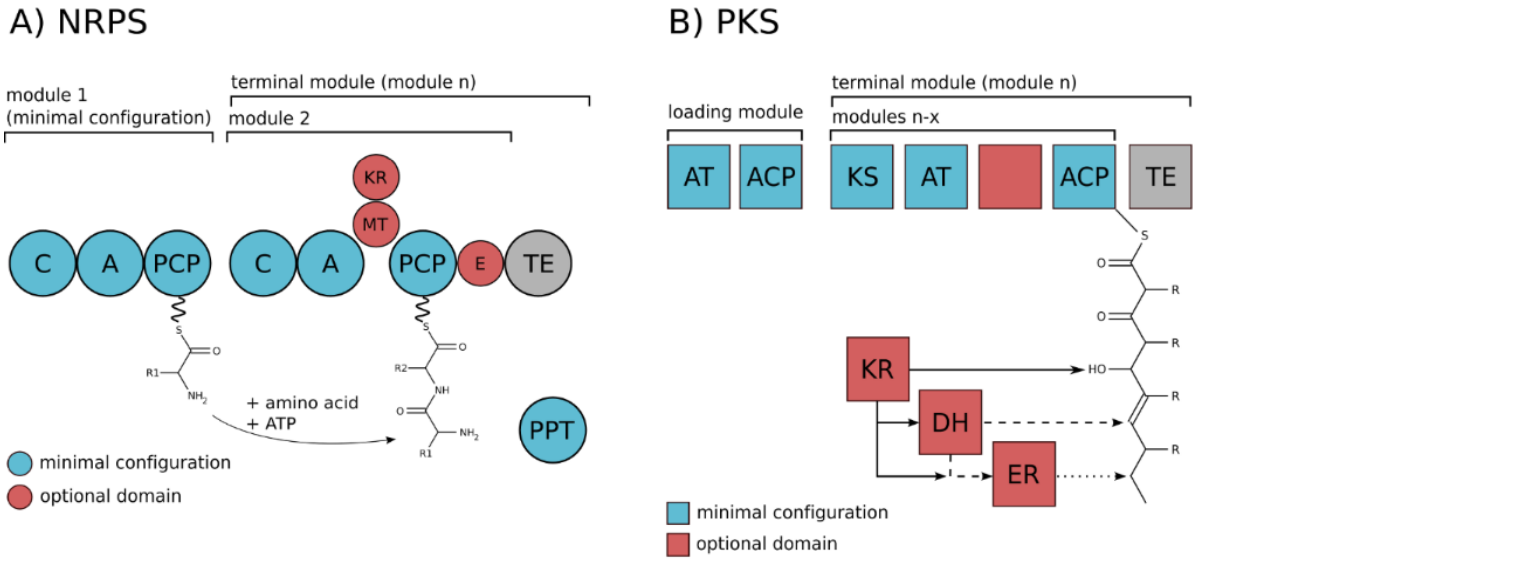
strain	clusters for secondary metabolism	observed secondary metabolites
S. avermictilis	37	13
S. coelicolor	30	16
Saccahropolyspora erythraea	27	3
Salinospora tropica	19	5

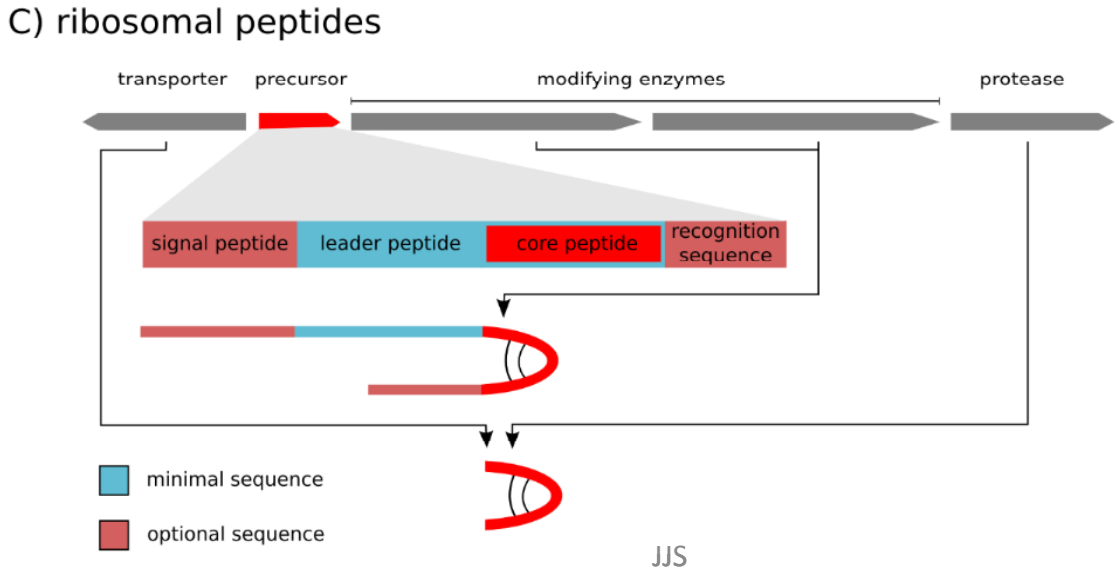
a part : **adaptive**

Nat.Prod.Rep. 2009, 26:1362

flexibility

Modular systems (gene in clusters)





Is it still possible to discover novel bioactive compounds from Actinomycetes ?

YES: potential and technologies

- Enormous potential of novel species in all habitats
- Potent methods for recognition of potential novel species, by chemical or genomic deconvolution
- Exploitation of talented strains More powerful chemical methods and databases
- Renaissance oif phenotypic screens and potent deconvolution of targets

Exploitation of natural biodiversity

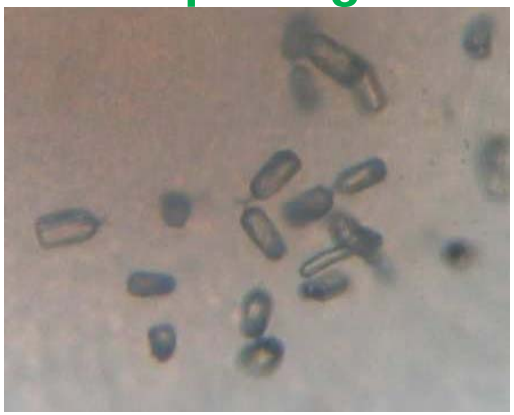
Targeted diversity of taxonomical units

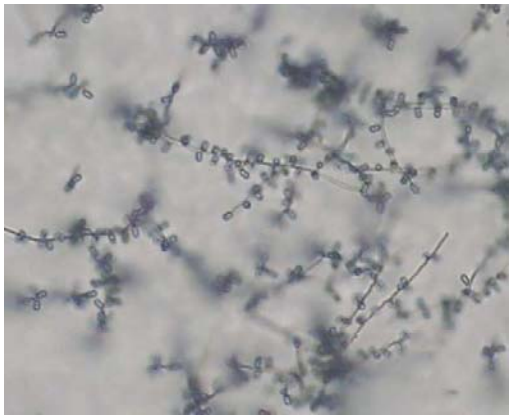
- Clear sampling strategy: not only exotic ecological niche(soils/ sediments/ plants)

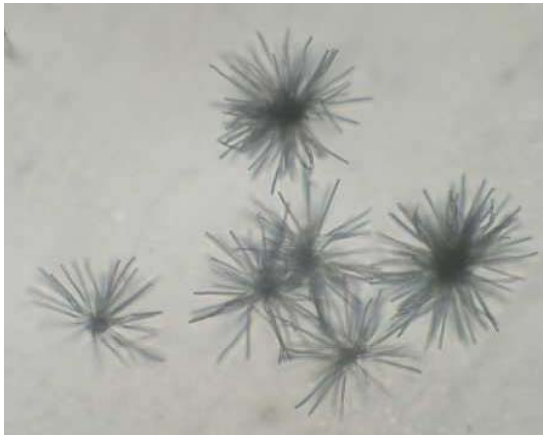
- Sample-treatment and selective media

- **Morphological observations and first grouping**

Key for success !







-16 s rRNA
-chemical profiling
-genomic

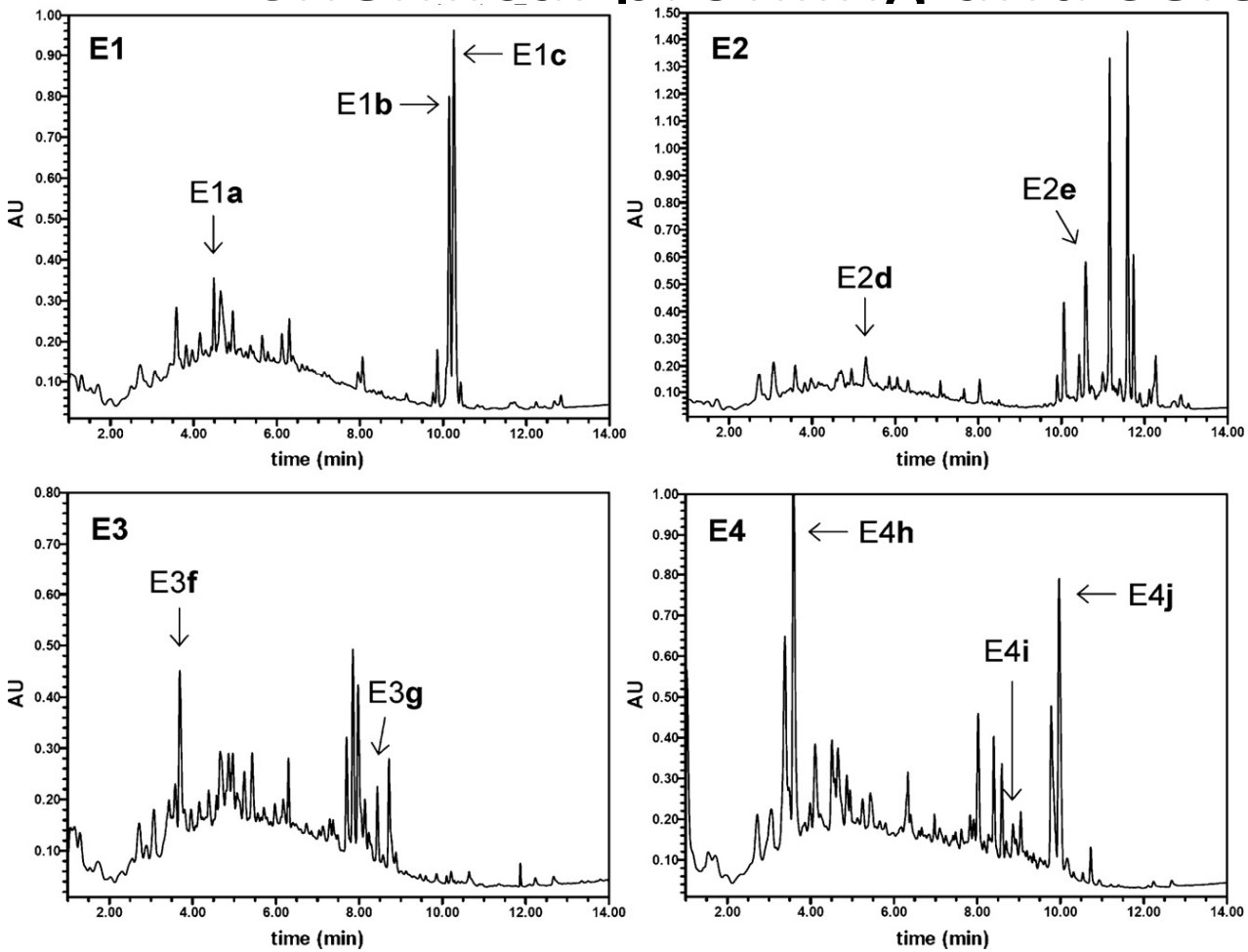
Putative novel species of Streptosporangiaceae from Thailand (terrestrial samples)

229 isolates were classified to 9 genera including 72 putative novel taxonomic units

genus	validated species	putative novel species
Herbidospora	5	9
Microbispora	7	8
Nonomuraea	32	11
Planomonospora	4	0
Planotetraspora	3	4
Streptosporangium	17	23

Chanwit Suriyachadkun, 2013

Chemical profiling and selection



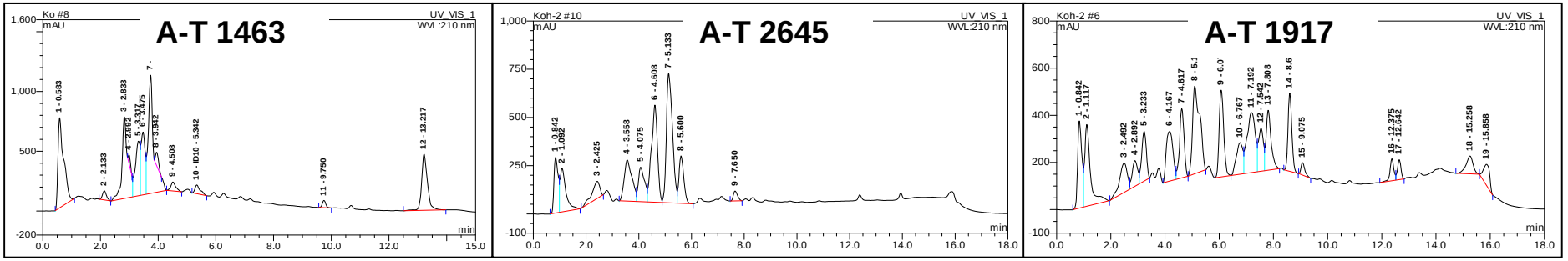
Database !

In each new « talented « strain novel compounds !

Journal of Chromatography A, 1217 (2010) 8016–8025

Examples of HPLC-profiles of strains belonging to one taxonomical unit

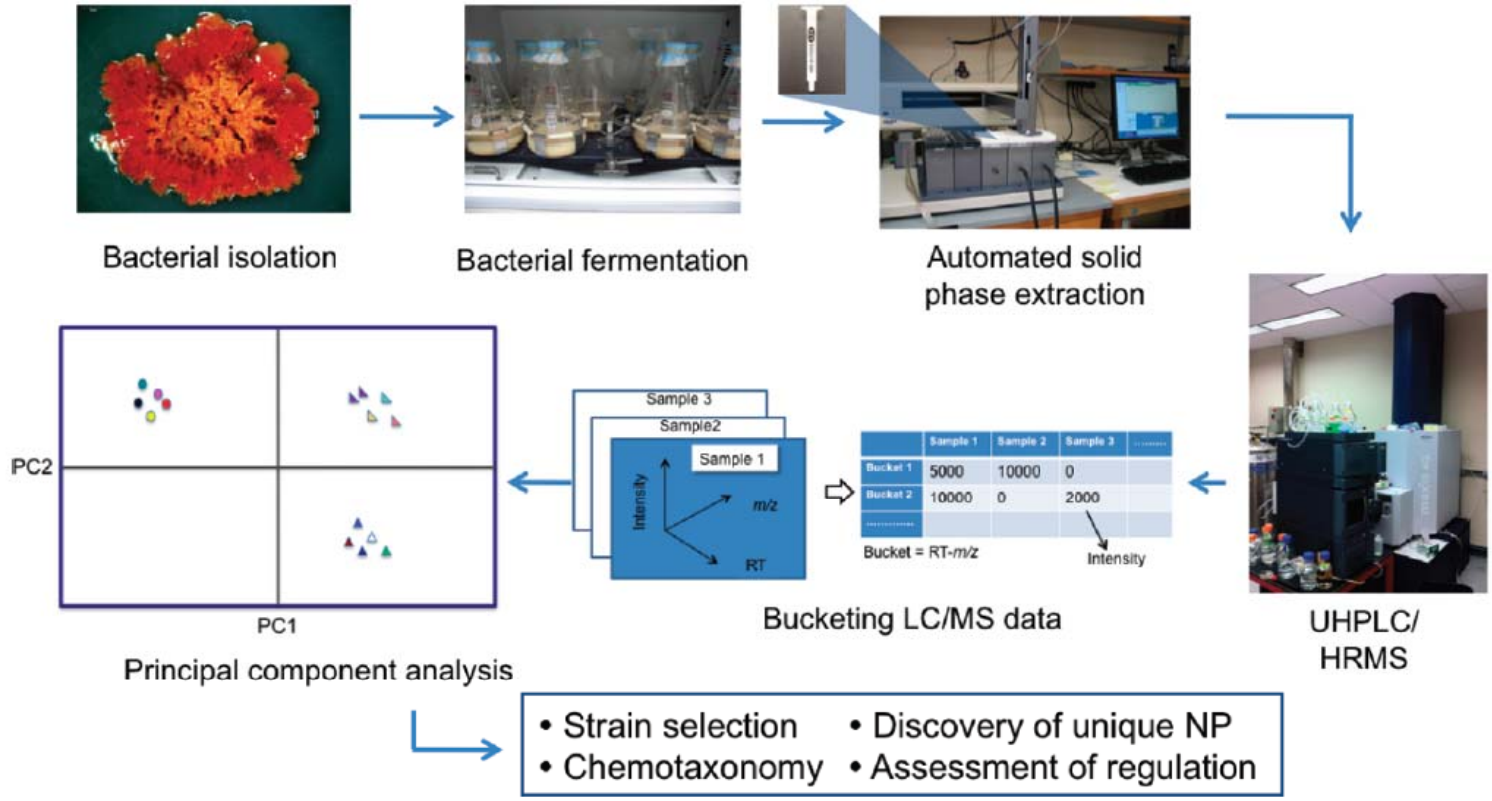
Microbispora group II (6)



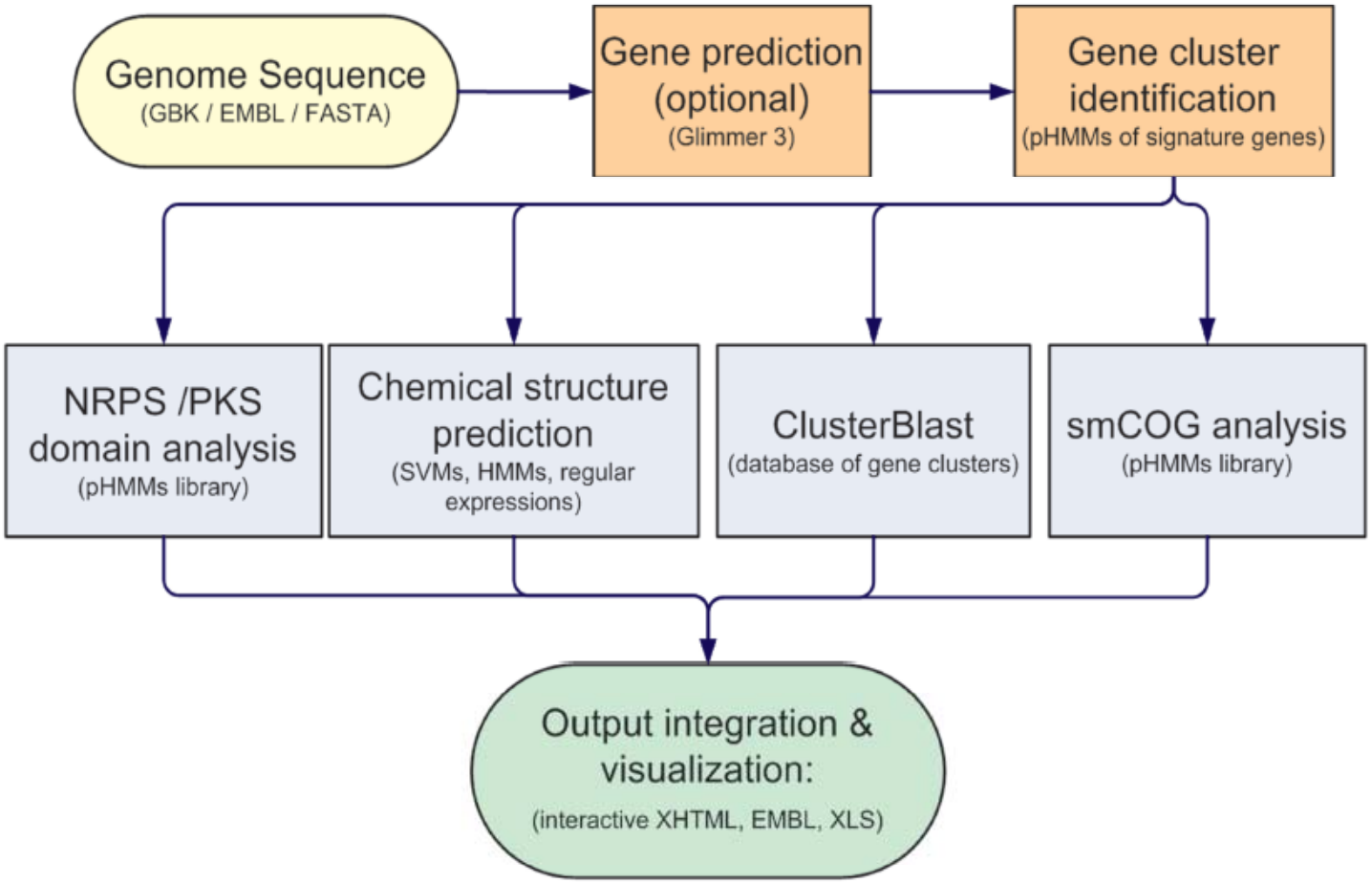
Chanwit Suriyachadkun, 2013

Prioritization by secondary **metabolomics**

untargeted LC/MS-based secondary metabolomics

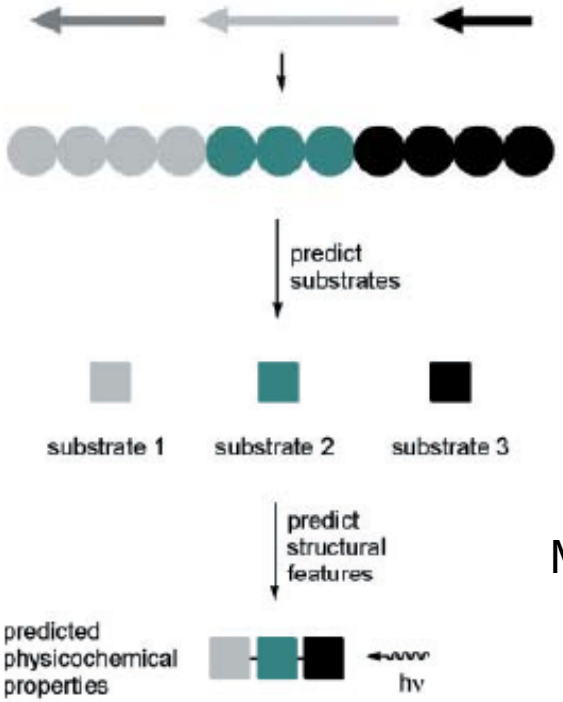


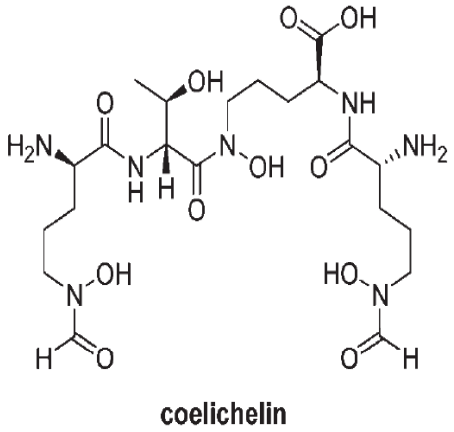
Hou et al., Anal. Chem. 2012, 84: 4277


Genome Sequence
(GBK / EMBL / FASTA)
Gene prediction
(optional)
(Glimmer 3)
Gene cluster
identification
(pHMMs of signature genes)
NRPS /PKS
domain analysis
(pHMMs library)
Chemical structure
prediction
(SVMs, HMMs, regular
expressions)
ClusterBlast
(database of gene clusters)
smCOG analysis
(pHMMs library)
Output integration &
visualization:
(interactive XHTML, EMBL, XLS)

Genomics screening for natural products

Use of sequence data to search for novel metabolites



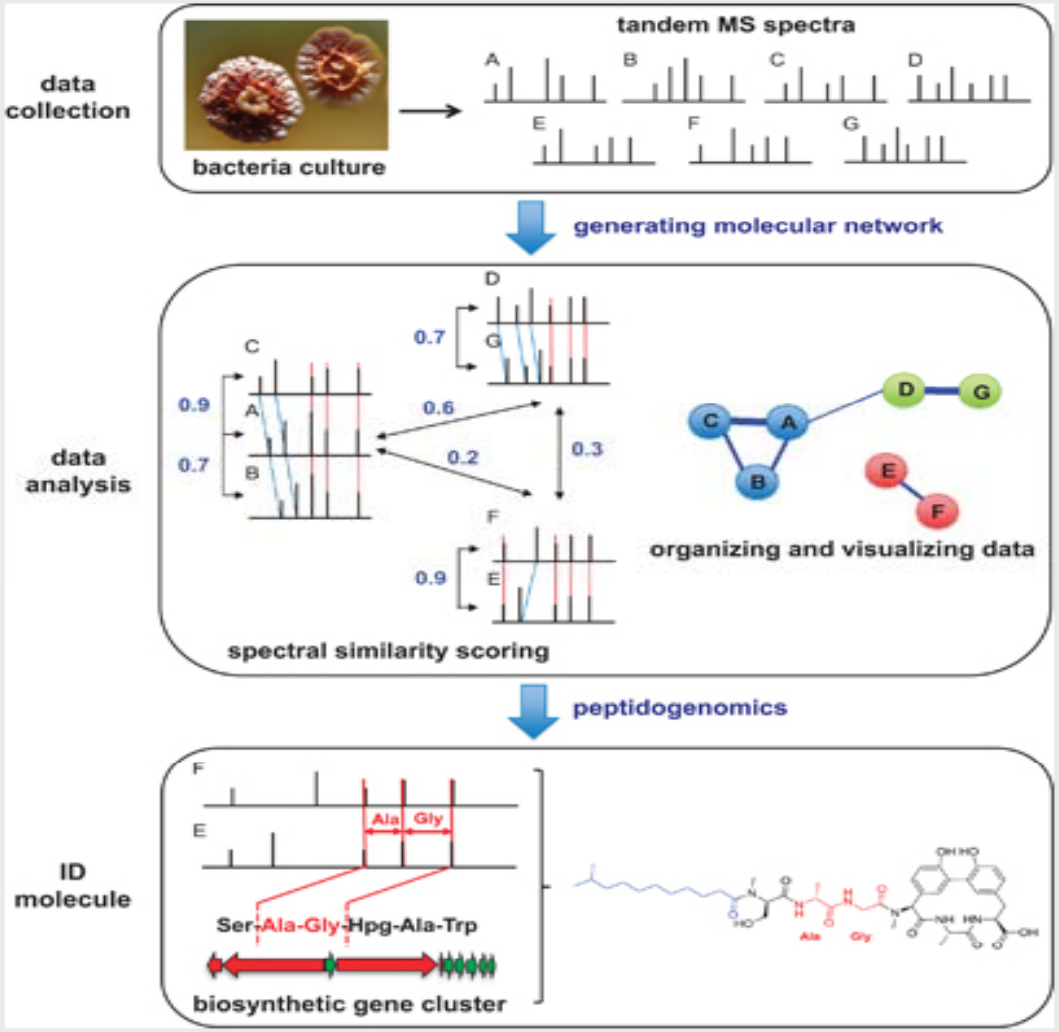


anti SMASH

Microbiology 2008, 154: 1555

scarcity of efficient procedures to connect genes to molecules ⟶ small fraction of secondary metabolomes investigated

Genome mining: molecular network

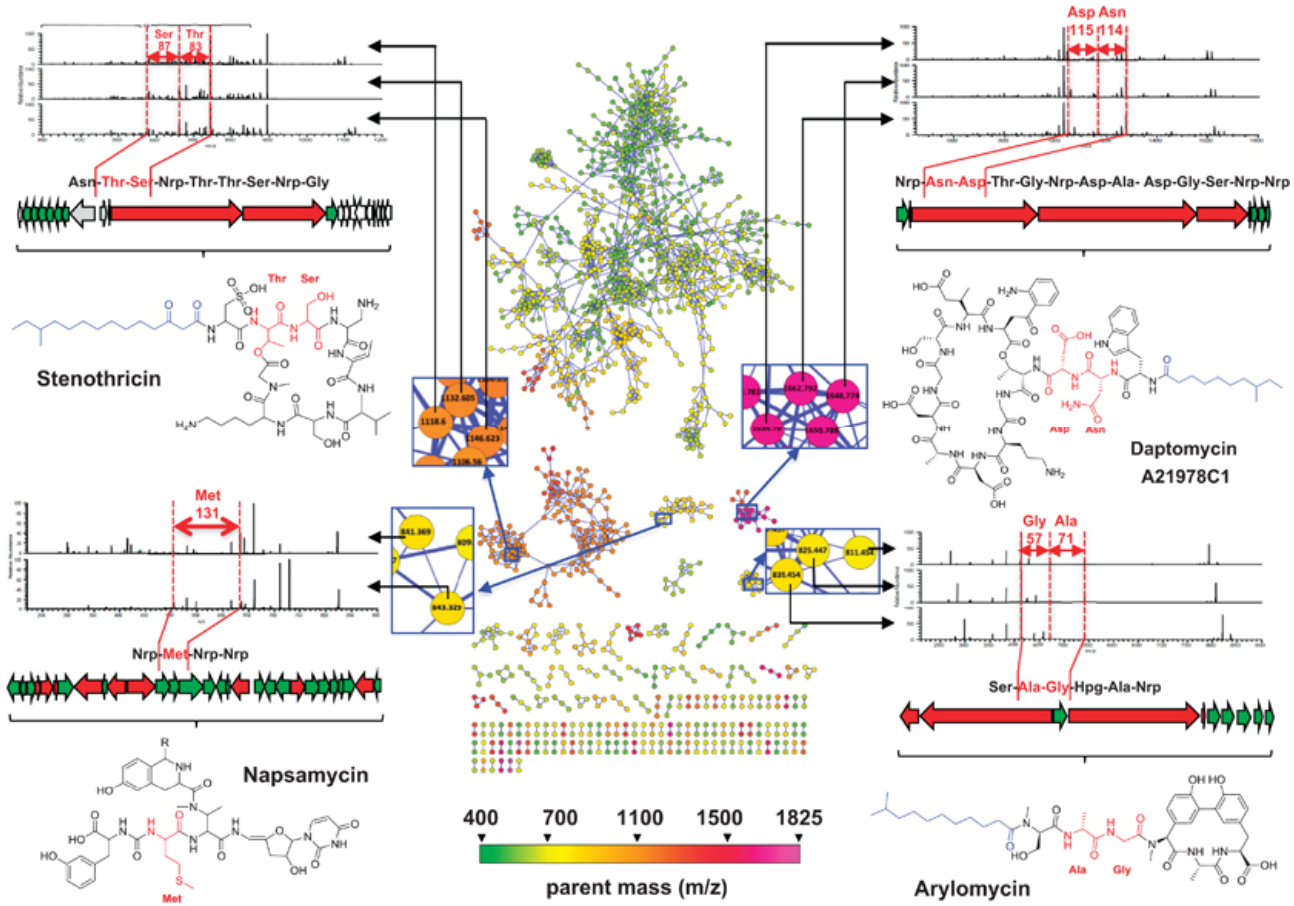


Streptomyces roseosporus
daptomycin producer

Liu et al., J. Antibiot. 2014, 67:99

Genome mining: molecular network

(MS / MS networking and peptidogenomics)



J. Antibiot. 2014, 67:99

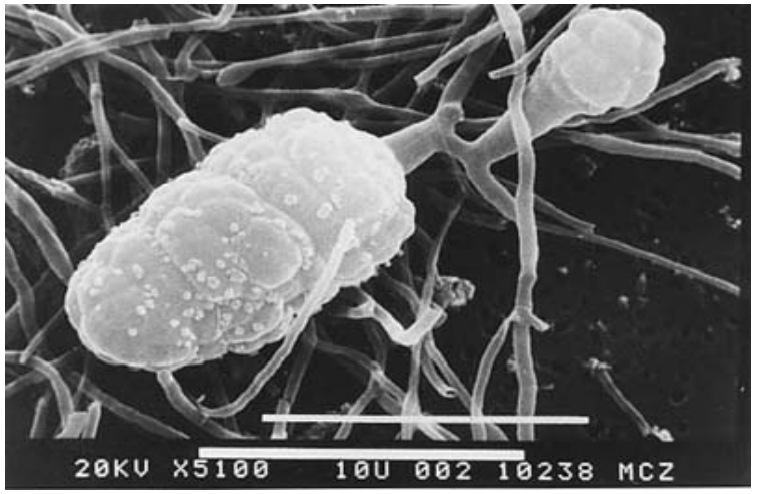
antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers

Features	antiSMASH 2.0
Open-source and stand-alone available	X
Covers bacteria, archaea and fungi	X
NRPS/PKS detection	X
NRPS/PKS detailed functional domain annotation	X
NRP/PK core structure prediction	X
Lantipeptide core structure prediction	X
Detection of other biosynthetic classes	X
Gene cluster border prediction	X
Comparative gene cluster analysis	X
Sub-cluster analysis	X
Prediction of putative novel gene cluster types	X
Protein sequence input	X
Nucleotide sequence input	X
Multi-contig input	X
PKS structural modeling	
NRPS/PKS domain phylogenomic analysis	(X)[a]

Detection of genera with attractive potential

Detection of unique or unusual
polyketide synthase type I (PKSI),
polyketide synthase type II (PKS II)
non-ribosomal peptide (NRPS)
hybrid NRPS-PKS

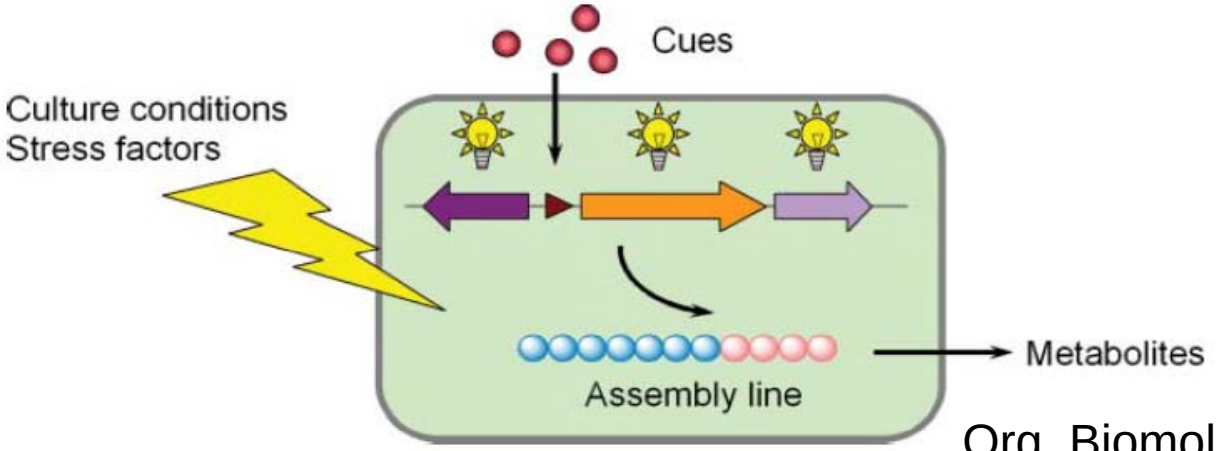
- ***Frankia***



vast majority of gene clusters (PKS) : unique.

BMC Genomics 2013, **14**:611

Exploitation of talented strains: physiological conditions



Org. Biomol.Chem. 2009, 7:1753

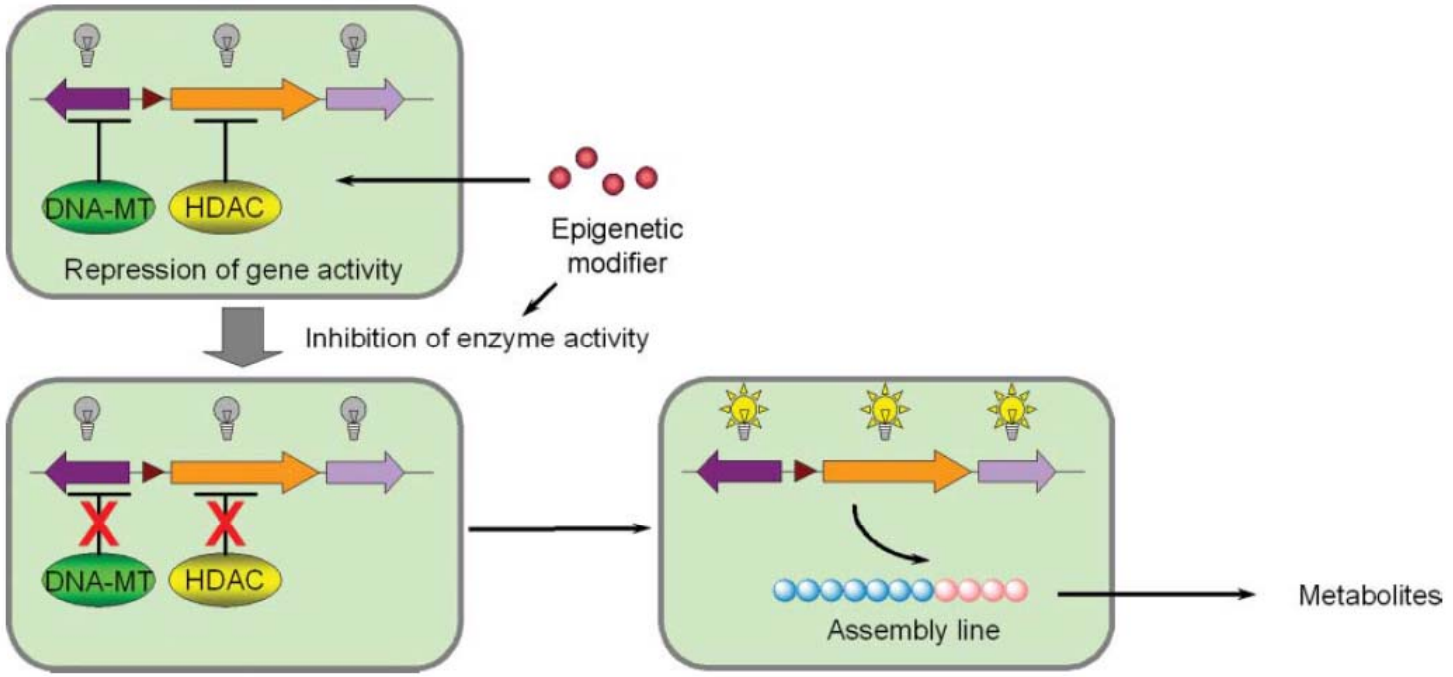
conditions (pH, C/N, temperature, aeration) ,

additions (DMSO, ions), stress (heavy metals,solvents,)

- activation of genes by scandium (Tanaka et al. 2010)

Applied Microbiology and Biotechnology2013, 97 : 87

Epigenic remodelling



Org. Biomol.Chem. 2009, 7:1753

DNA methyltransferase and histonedeacetylase inhibitors

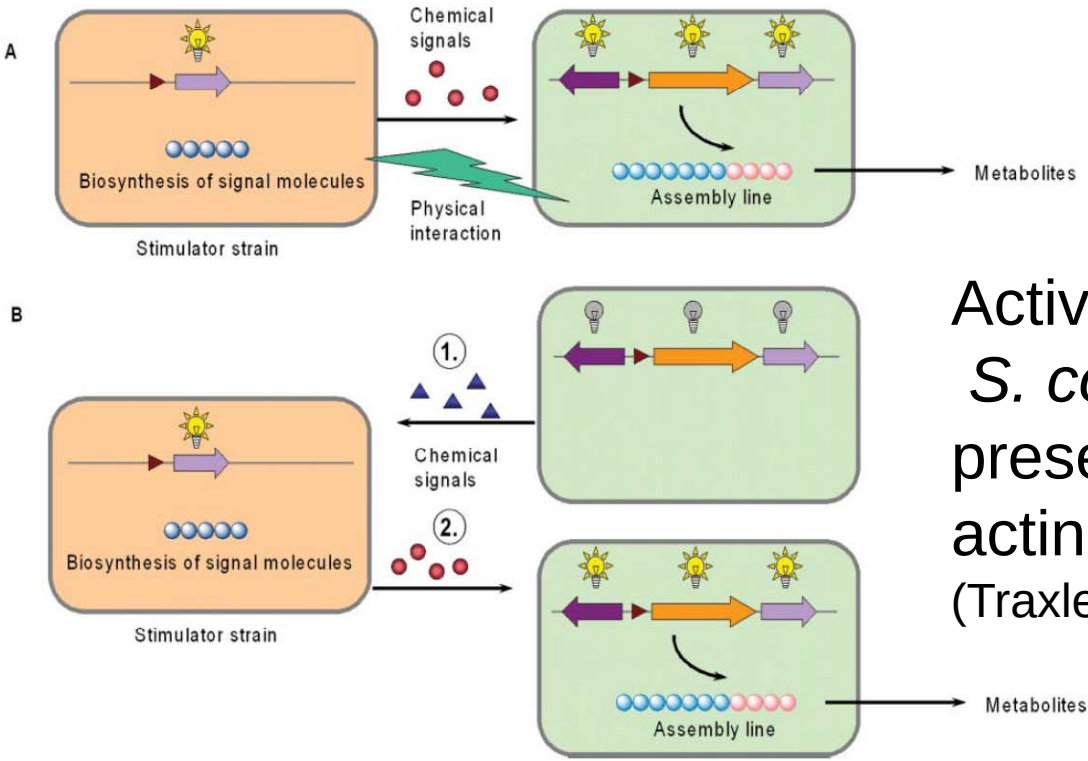
Sodium butyrate as an HDAC

5-azacycline

goadsporin

enzyme inhibitors such as tricyclazole

Combined cultures

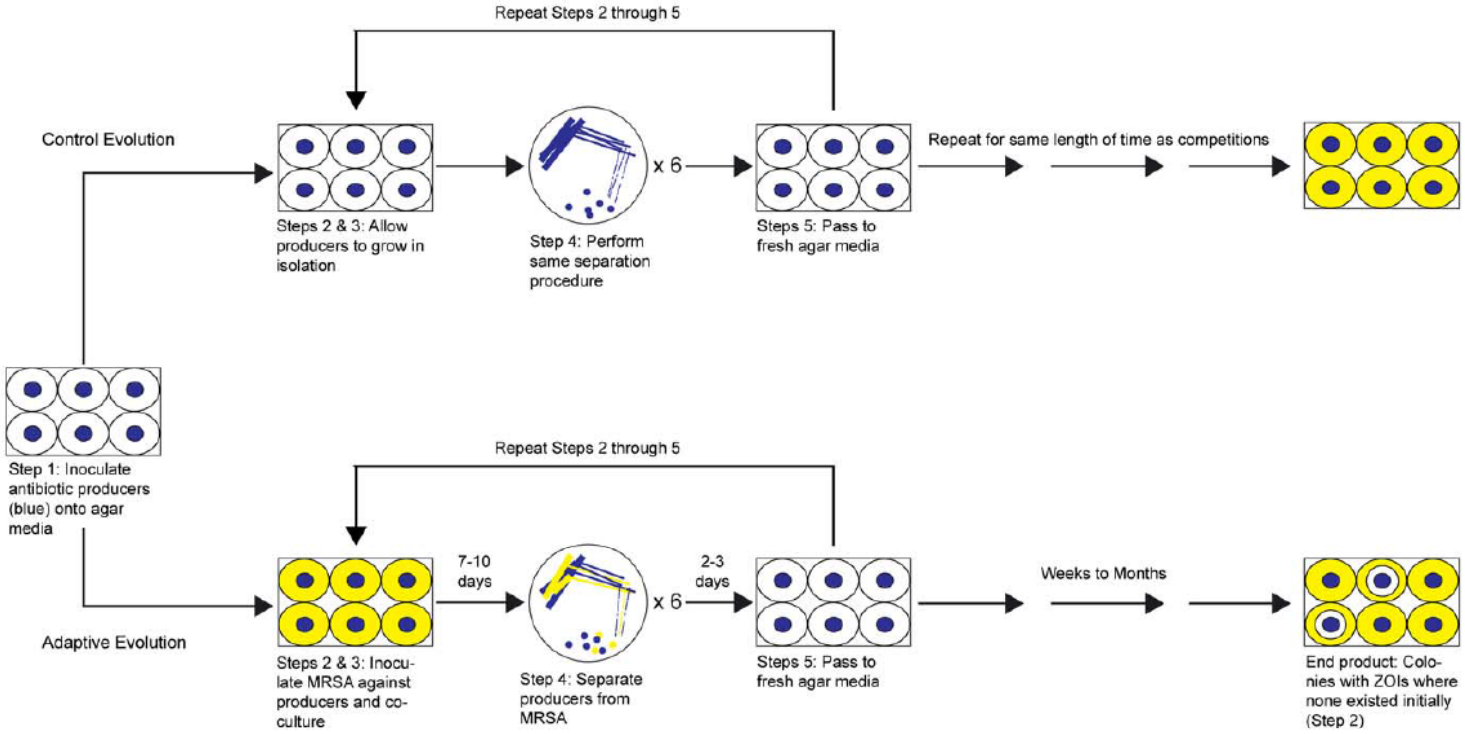


Activation of genes in *S. coelicolor* by the presence of other actinomycetes
(Traxler et al. 2013, mBio 4)

e.g. **mycolic acid containing** bacteria

- induction of new metabolites in 37 %
- increase in 55 % (Onaka et al., 2011)

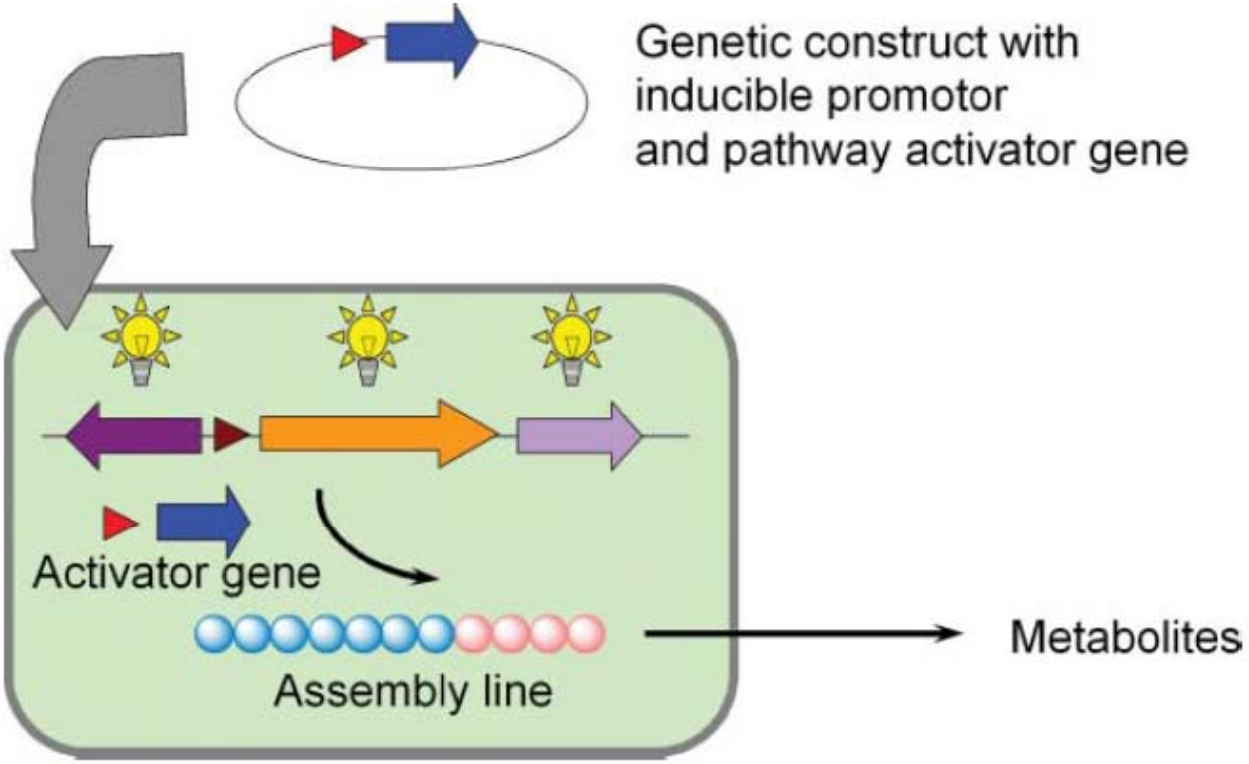
Adaptive Laboratory Evolution



… or experiment in soil

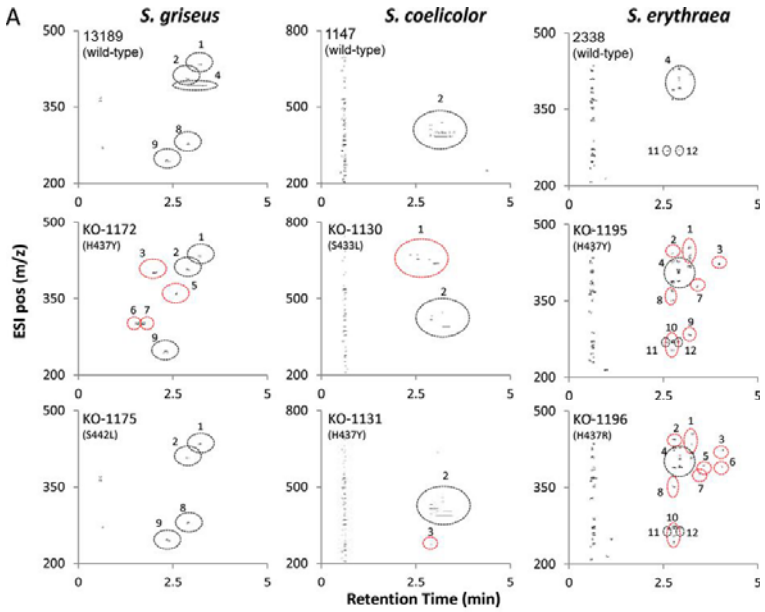
Eg : holomycine

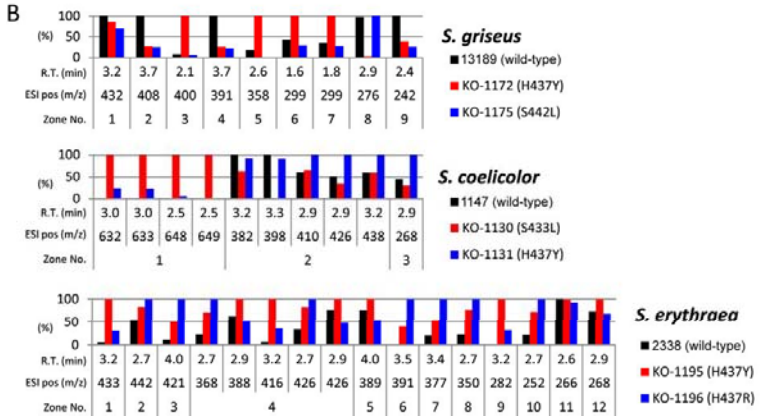
Gene activation



Org. Biomol.Chem. 2009, 7:1753

Non specific activation: rifampin resistance (*rpoB*) mutations



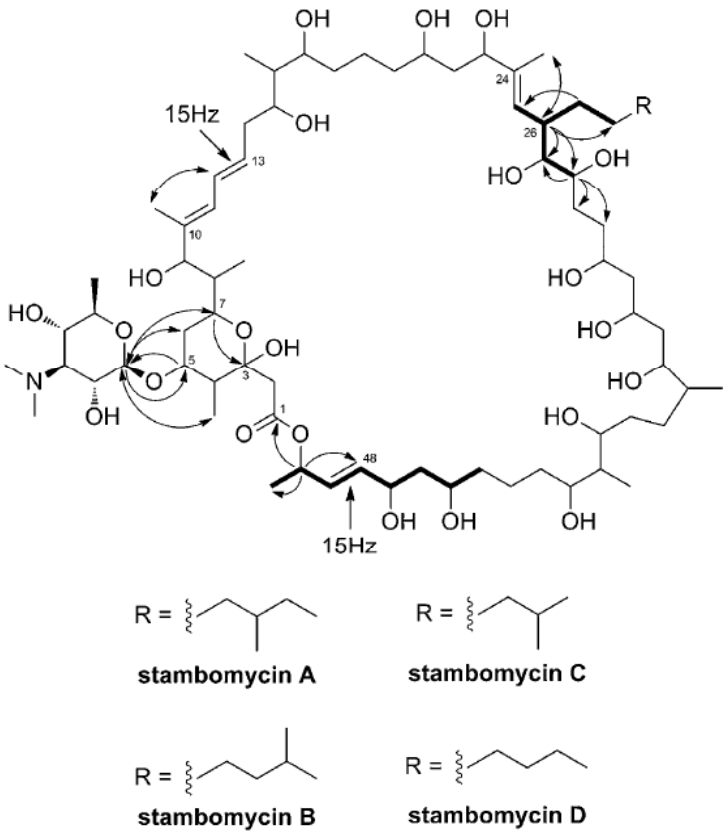


"ribosome engineering"

Analysis of the metabolite profile derived from the rpoB mutants.

Tanaka Y et al. J. Bacteriol. 2013,195:2959

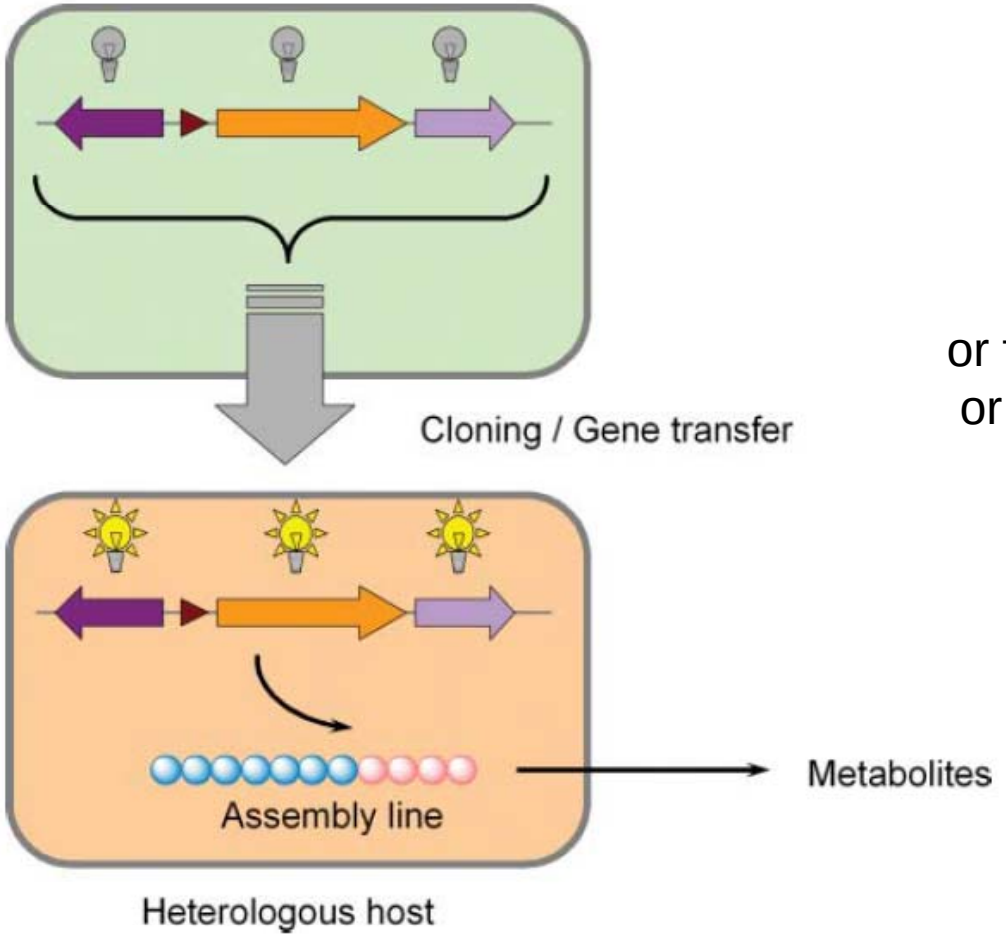
Identification and targeted activation gene cluster



- *Streptomyces ambofaciens*
- Gene cluster 25 genes = 25 modules, 150 kb
- Not expressed
- Constitutive expression og a protein LAL family triggered the expression of the genes (cloning, strong promotor)
- 51-membered glycolated macrolide

Proc Natl Acad Sci U S A. 2011,108: 6258

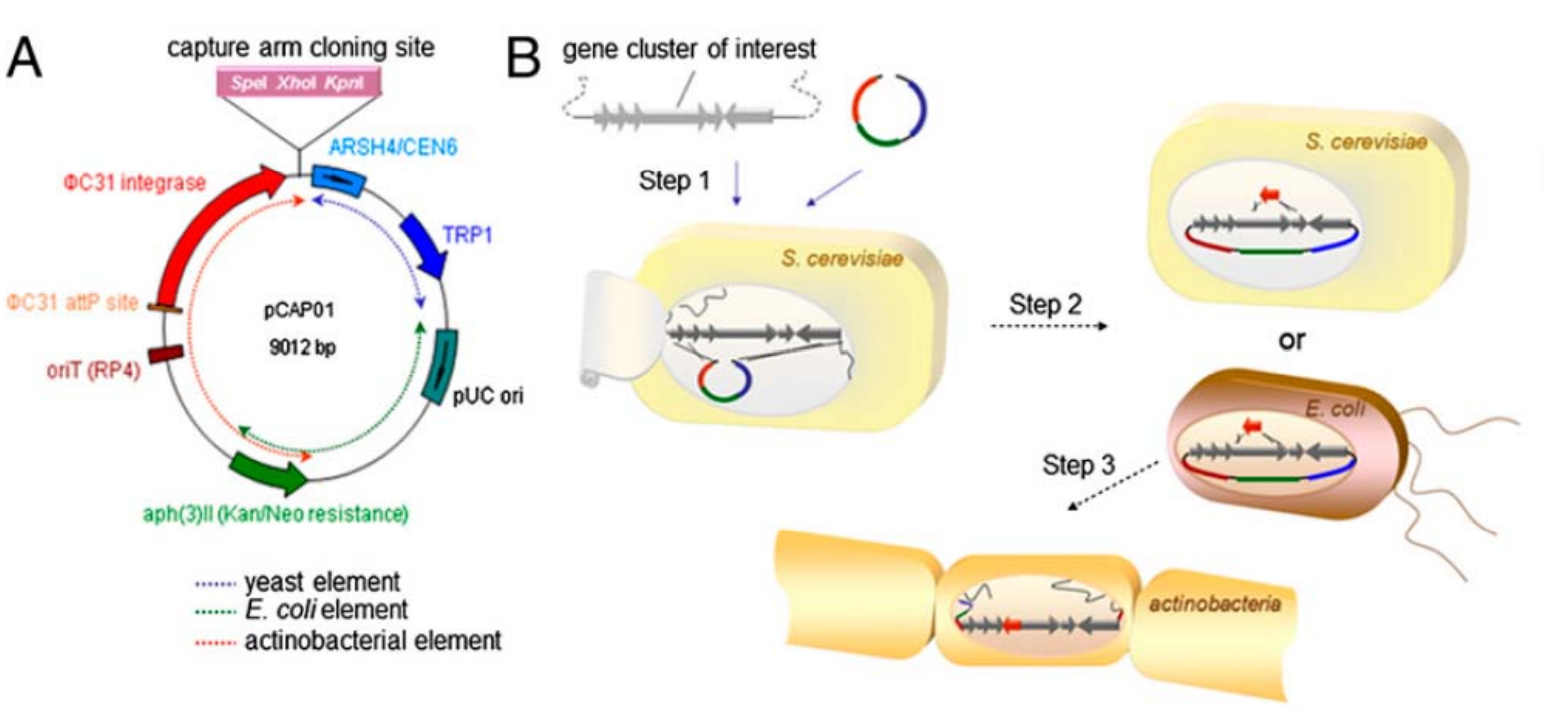
Heterologous expression



or from soil
or from sponges,…

Org. Biomol.Chem. 2009, 7:1753

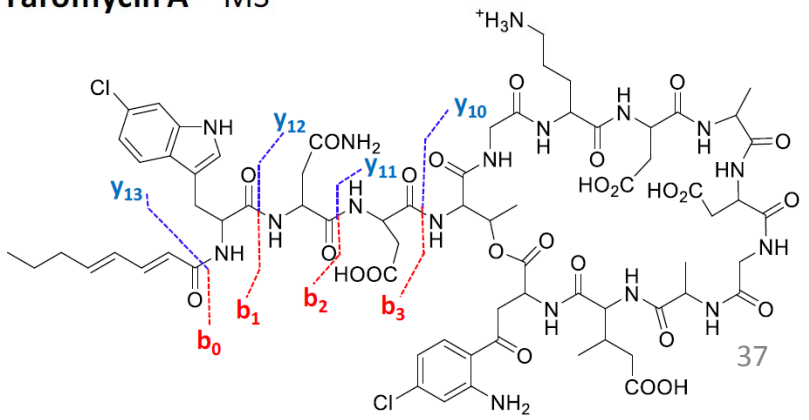
Transformation-associated recombination (TAR)

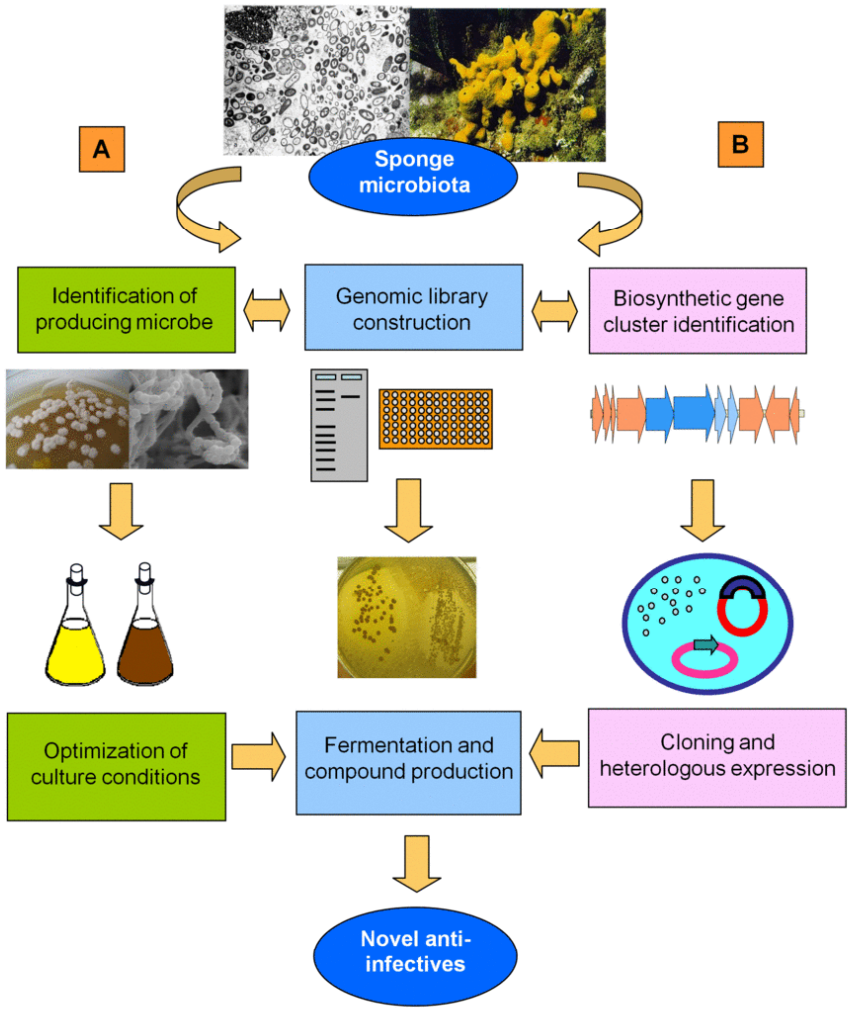


From uncharacterized gene collections
directly clone and express
orphan biosynthetic gene clusters

Yamanaka et al. PNAS 2014, 111 :1957

Taromycin A – MS^2



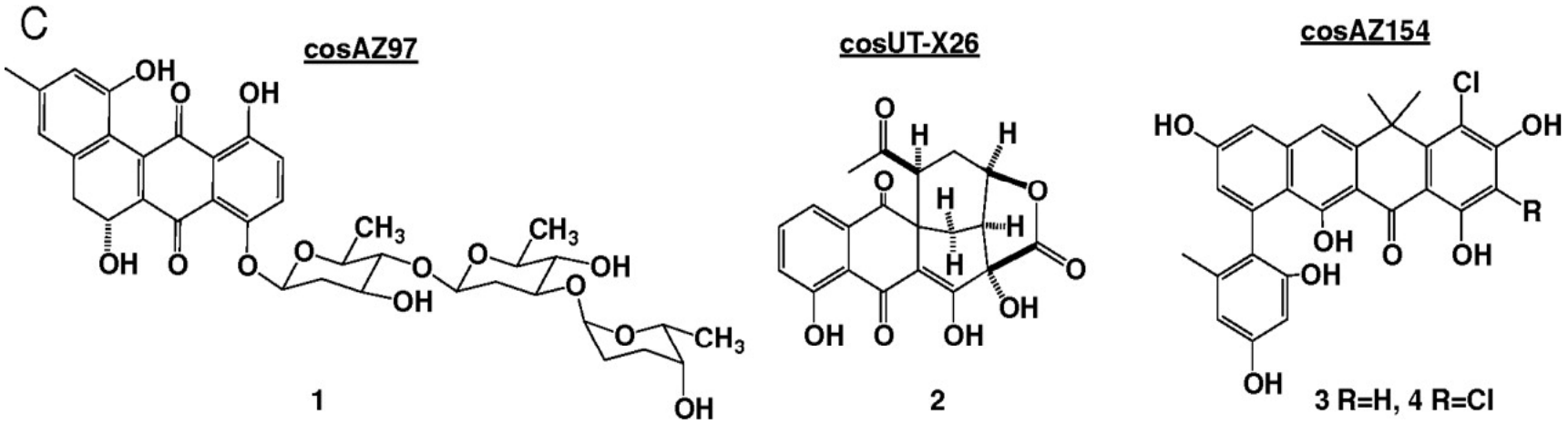


Culture-dependent (A) and culture-independent (B) strategies to access the biosynthetic potential of sponge-associated microorganisms

Ute Hentschel-Humeida, 2013

Recombinant environmental libraries

clone “shotgun” environmental DNA library by using an *Escherichia coli-Streptomyces lividans* shuttle cosmid vector and DNA inserts from microbes derived directly from soil.



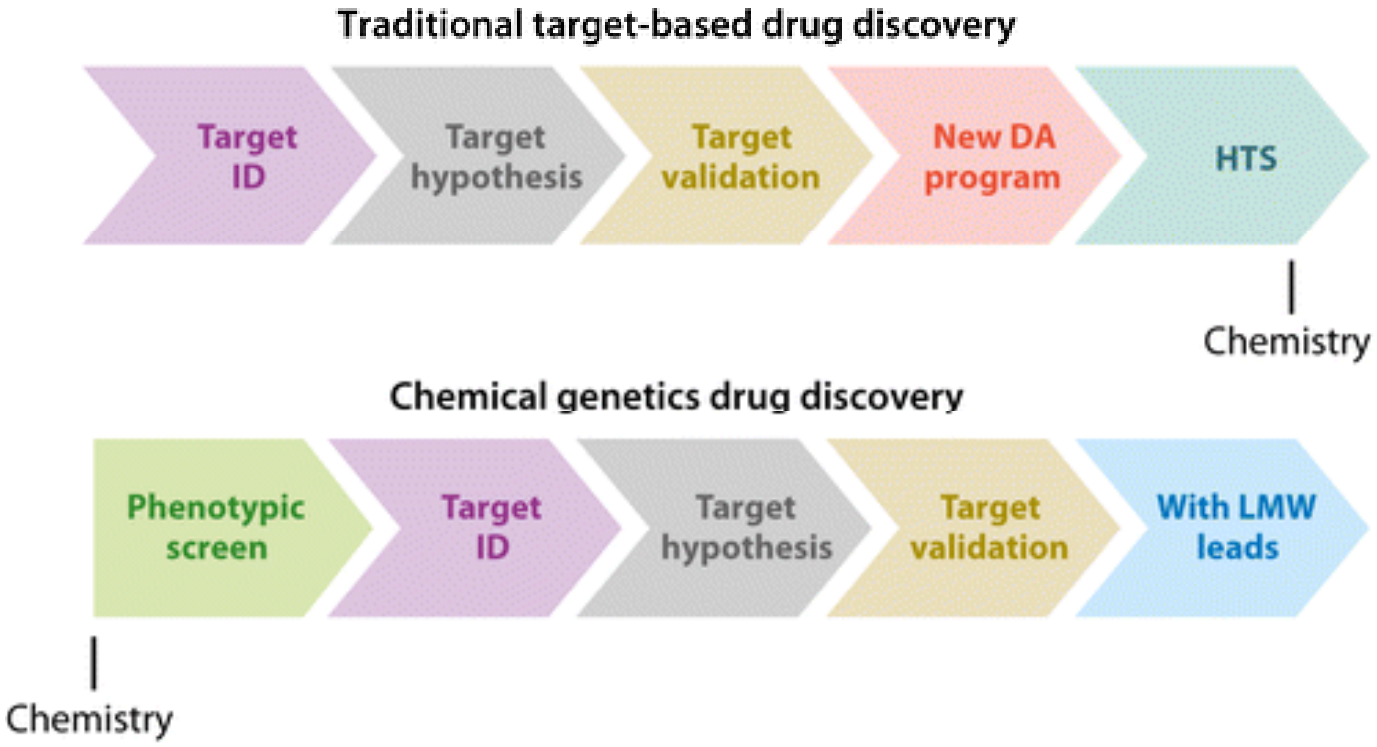
Proc Natl Acad Sci U S A. 2011, 108:12629

Generation of New Analogs by Genetic Engineering

Natural product library and database

- The building up of a repository of defined fractions and better of purified, characterized compounds is mandatory.
- The data should be easily accessible for generating reports and data mining.
- The data schema and program functions should facilitate the dereplication of natural products, give information on taxonomy and genetic potential of the microbial strains

Screening assays:2 strategies



Cong F, et al. 2012.
Annu. Rev. Pharmacol. Toxicol. 52:57–78

Phenotypic versus target-based approaches

Phenotypic

- No need of prior knowledge of the molecular mode of action
- Activity in phenotypic assays bear the change to be translated more effectively into therapeutic impact

- Assays with lower throughput
- Commitment of drug candidate optimization with phenotypic assay
- Deduction of putative targets is time intensive

Natural products : tools demonstrating specific pathways in diseases

Target-based

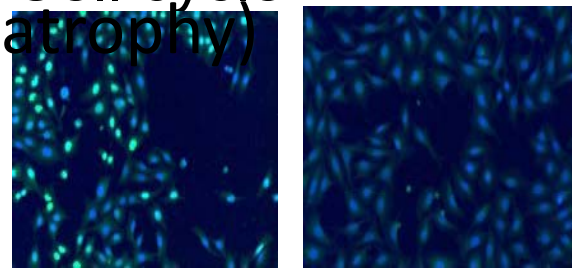
- Application of molecular and chemical knowledge
- Small-molecule screening and biological approach with monoclonal antibodies
- High throughput screening assays

- Specific hypothesis is not relevant to disease pathogenesis

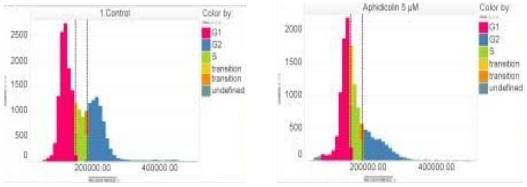
Drug discovery through phenotypic assays

- Cell cycle and cell proliferation in cancer progression
- Morphological changes of cell organelles like mitochondria are involved in a plethoria of diseases
- Cell morphology changes linked to muscle diseases (atrophy)

Cell cycle

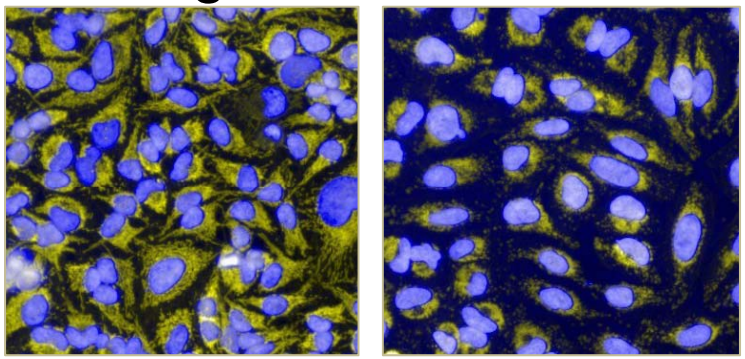


DMSO control Aphidicolin 5 uM



U2OS cells, Hoechst (blue), EdU (green)

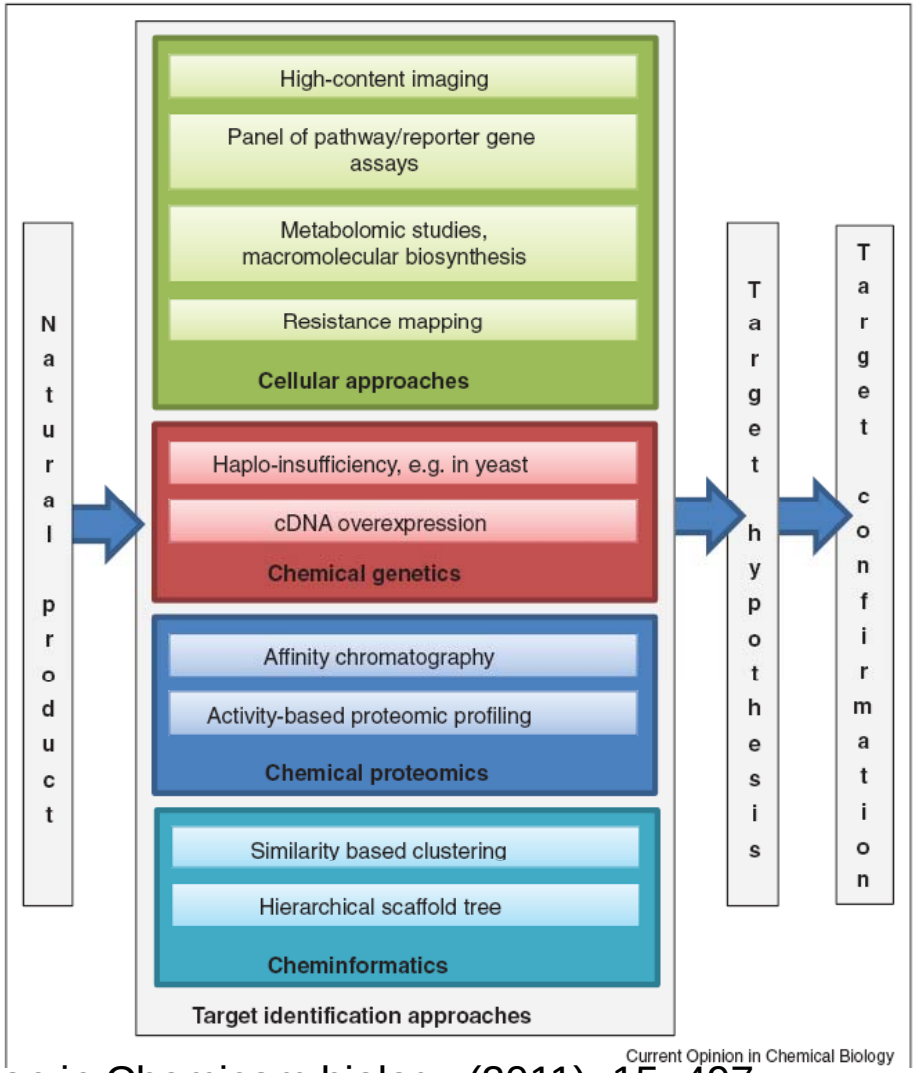
Cell organelle



DMSO control Mito decoupler, 10 uM

HeLa cells, Hoechst (blue), Mitochondrial marker (green)

Target identification for natural products using an array of different technologies.



Curr Opinion in Chemicam biology (2011); 15: 497

conclusions

- Actinomycetes secondary metabolites is a source of various bioactive compounds and of pathfinders
- Wild strains remain a main source of natural products
- Cultures conditions, co-cultivation, genetic methods allow the increase of titers ans more important the epression of cryptic genes
- Various methods are available to characterize strains, taxonomical position and metabome
- Novel assay technologies allow the discovery of novel tqrgets
- The potential is huge. Expertise and equipment are required.