Bioprospecção de Produtos Naturais

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Bioprospecção de Bioprodutos

Que são Bioprodutos?

Principais produtores

Funções biológicas

Produtos naturais= metabólitos, produtos bioativos

sintetizados por microrganismos

Plantas, animais

Desenvolvimento de medicamentos biorremediação, bioinseticidas, bioherbicidas

Metabolismo Primário: aminoácidos-proteínas-vitaminas-ácidos nucleicos-hormonios

Intermediarios

Ciclo Total

Metabolismo Secundario: terpenos/ polícetideos/melanina/aminoglicosídeos/ B-lactámicos

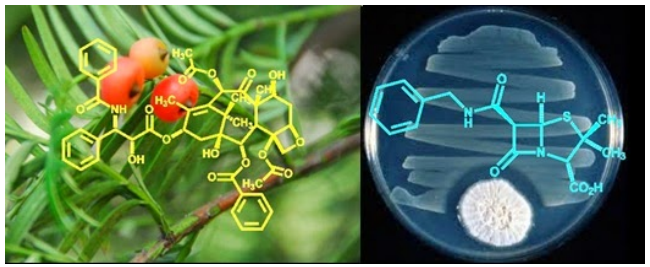
Ciclo parcial-fase

Produtos naturais

Compostos de baixo peso molecular

Antibióticos
Antifúngicos
Imunossupresores
Surfactantes
Antiparasitários
Anti-inflamatórios,
Antitumurais
Antivirais
Herbicidas
Pigmentos

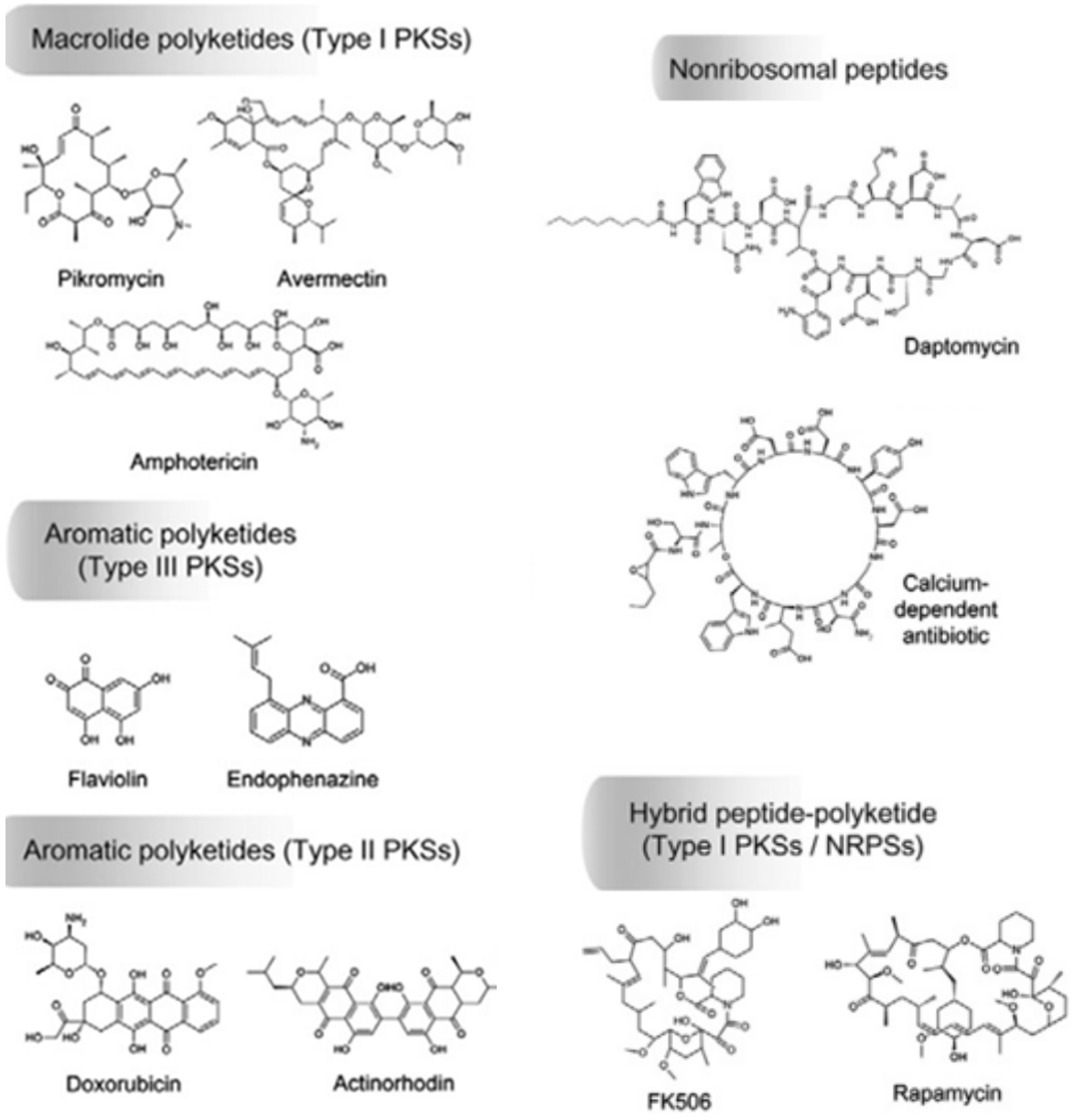
80% agentes antitumorais e 50% dos fármacos aprovados pela FDA são produtos naturais ou derivados.



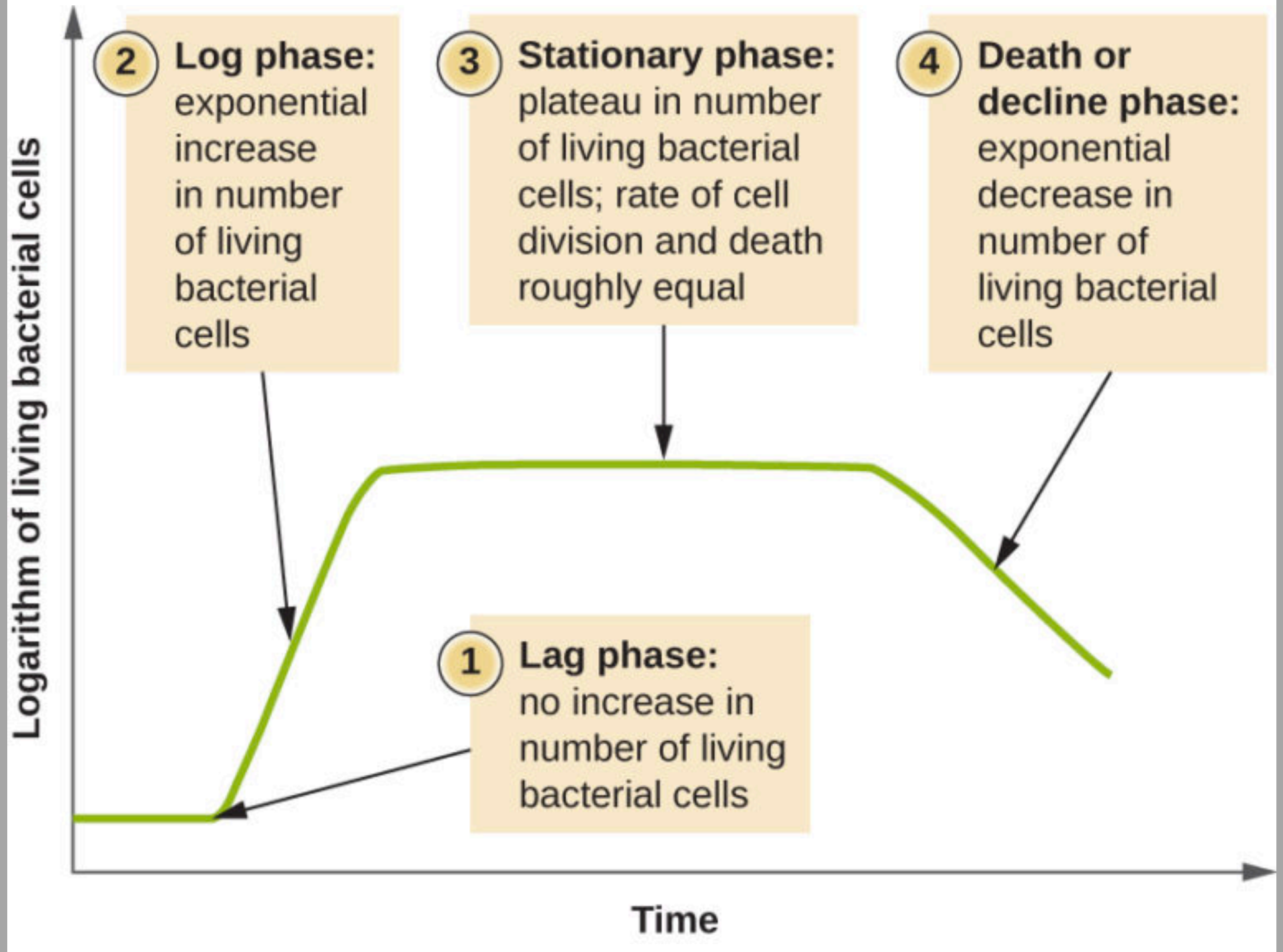
Grande diversidade de moléculas

Policetídeos
Peptídeos no ribosomaiss
Híbridos
Aminoglicosídeos
Terpenos
Sideróforos
Lantibióticos
Bacteriocinas
Butirolactonas
Ectoínas

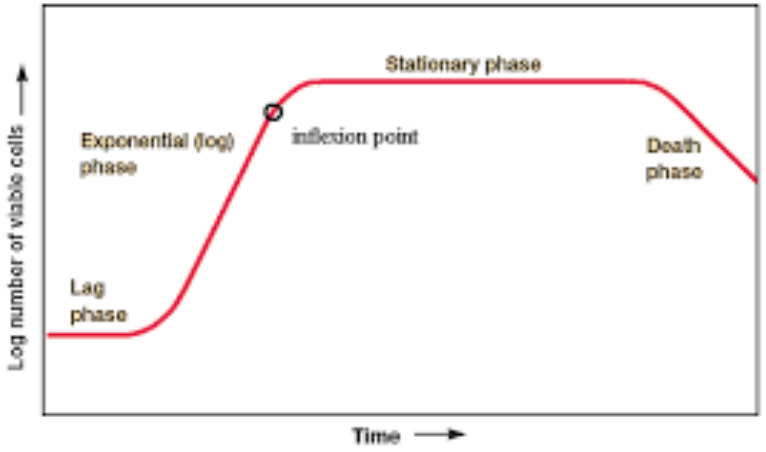
Necessidade de caracterizar novos *scaffolds* químicos



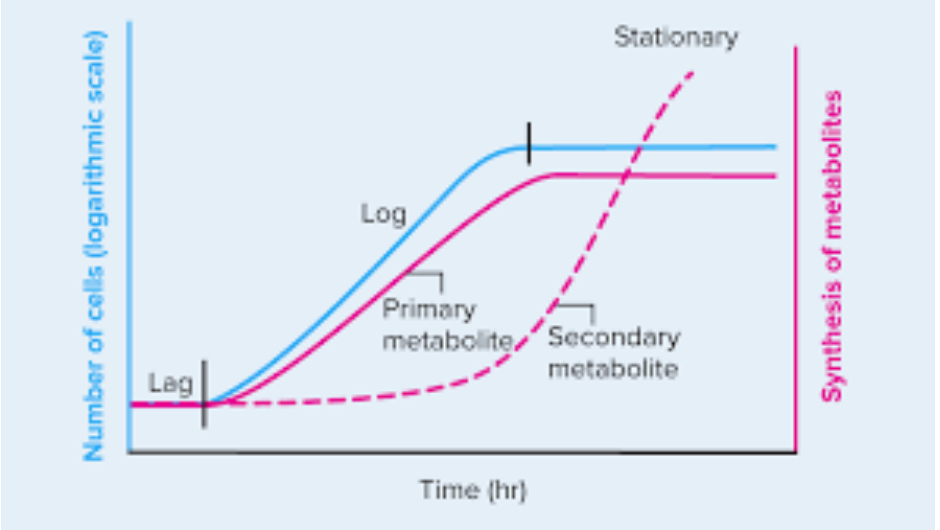
Adaptado de Hwang et al, 2014


Logarithm of living bacterial cells
2 Log phase: exponential increase in number of living bacterial cells
3 Stationary phase: plateau in number of living bacterial cells; rate of cell division and death roughly equal
4 Death or decline phase: exponential decrease in number of living bacterial cells
1 Lag phase: no increase in number of living bacterial cells
Time

Primary and secondary metabolism



Microbial Growth Curve in a Closed System.



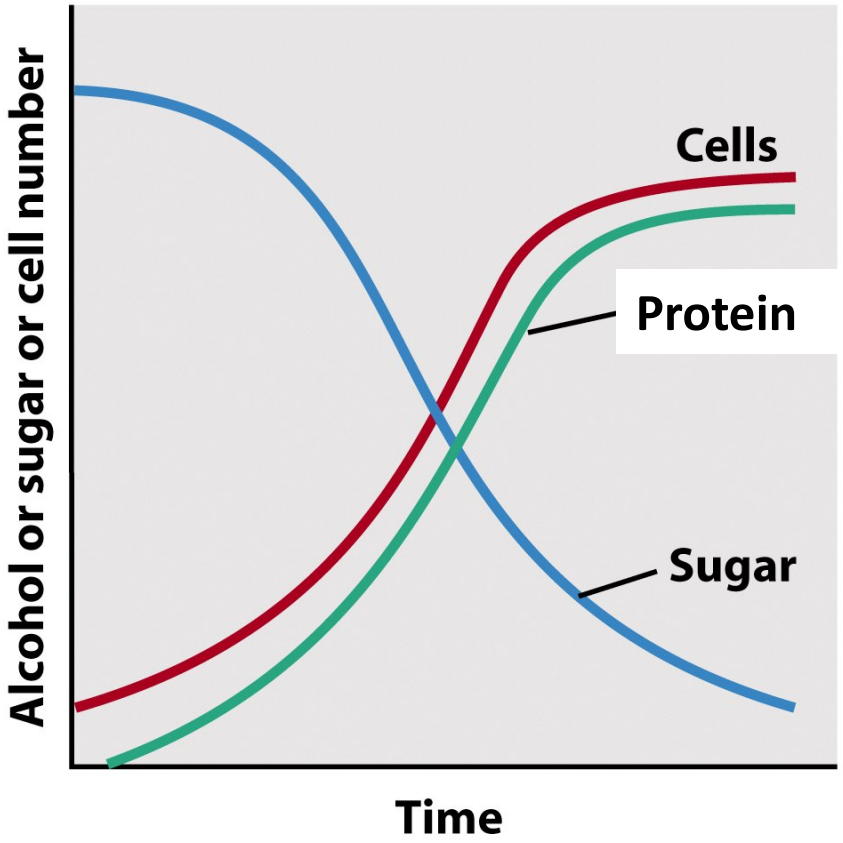


Figure 30-2a Brock Biology of Microorganisms 11/e

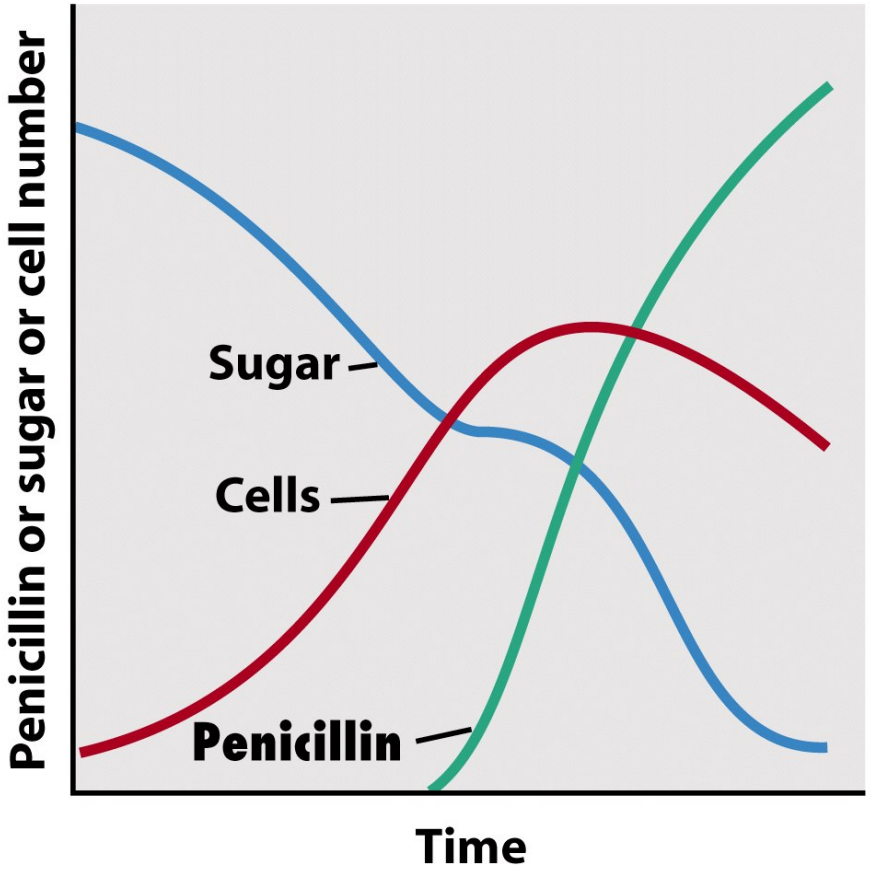
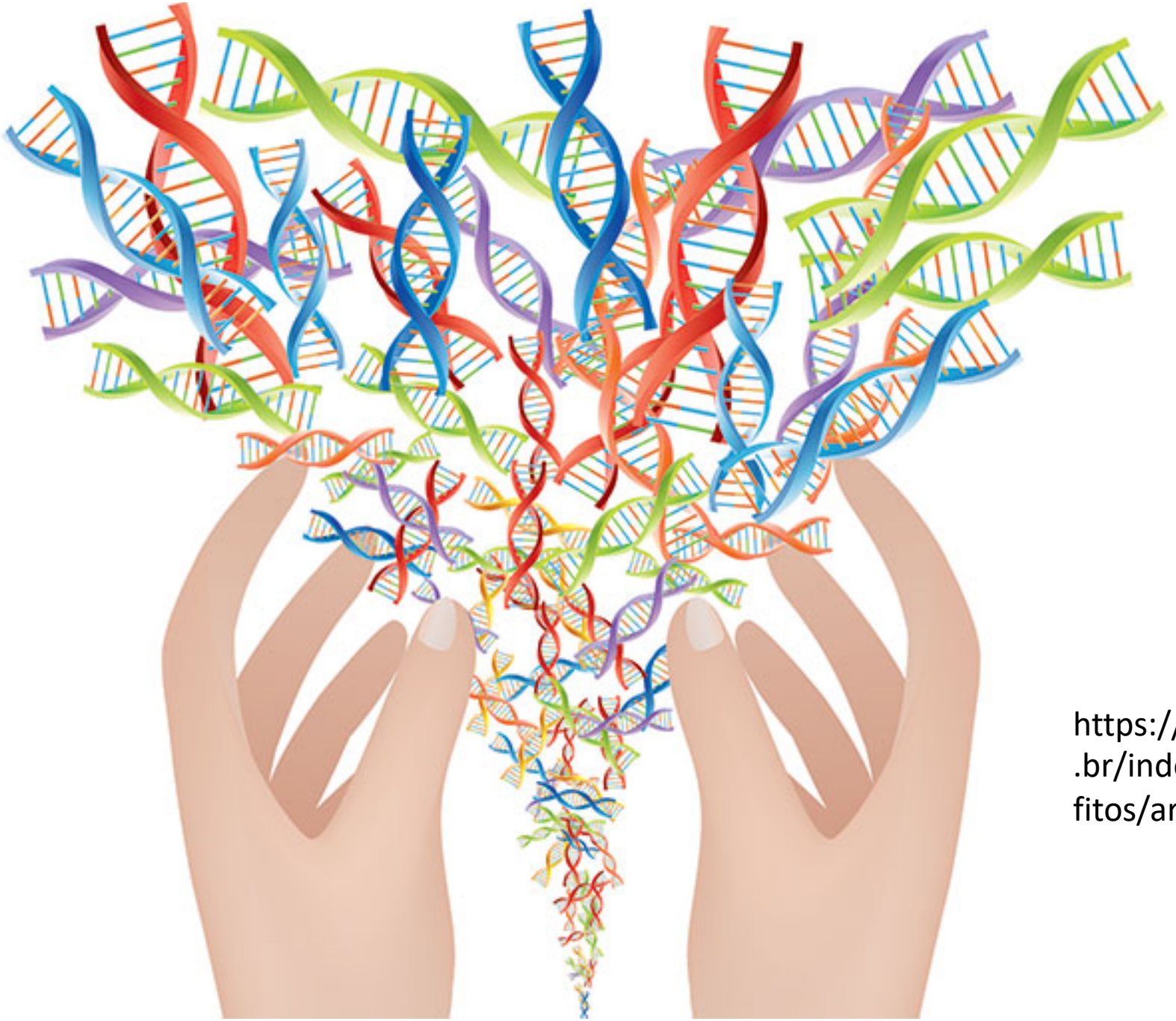


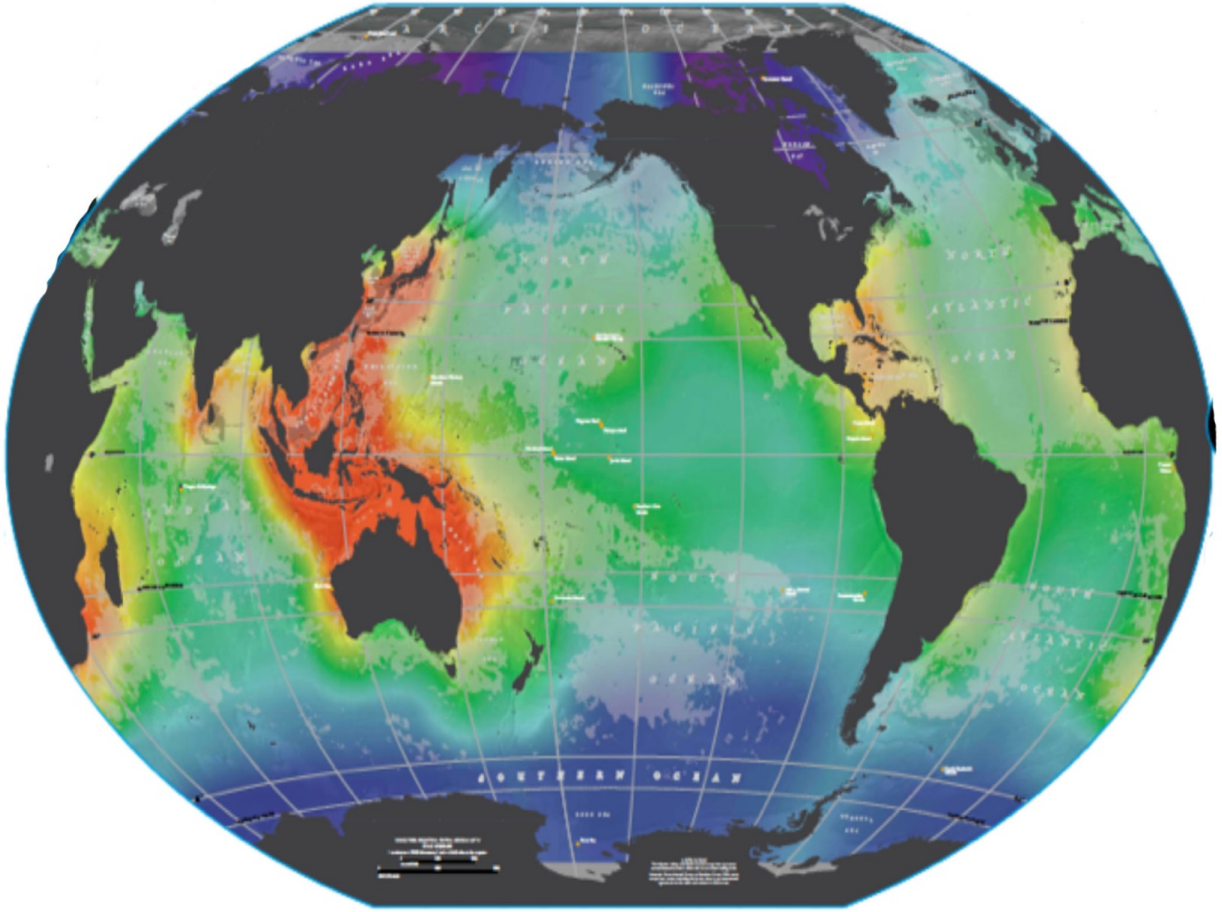
Figure 30-2b Brock Biology of Microorganisms 11/e

Primary and secondary metabolism



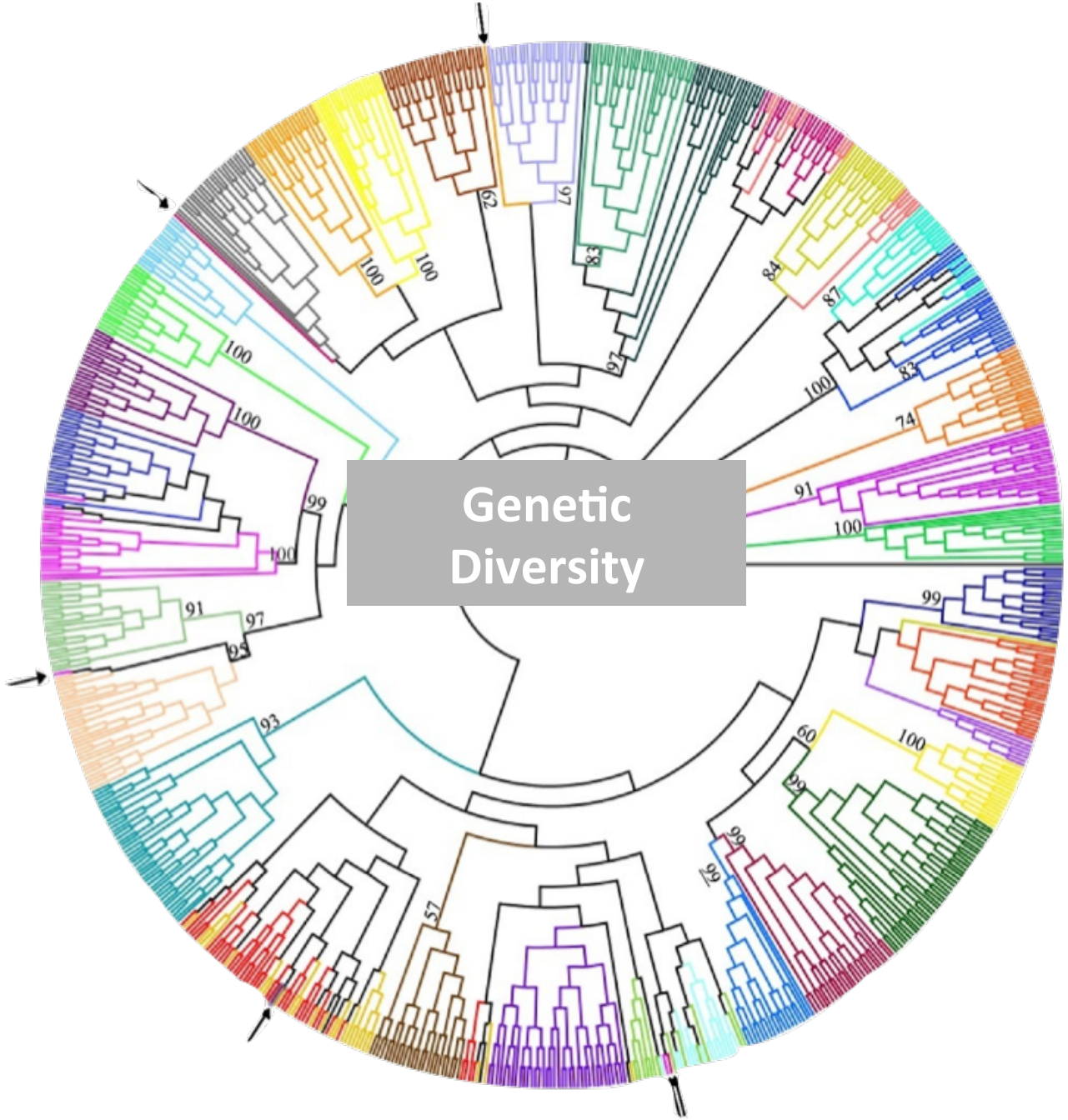
https://revistafitos.far.fiocruz.br/index.php/revista-fitos/article/view/1313/956

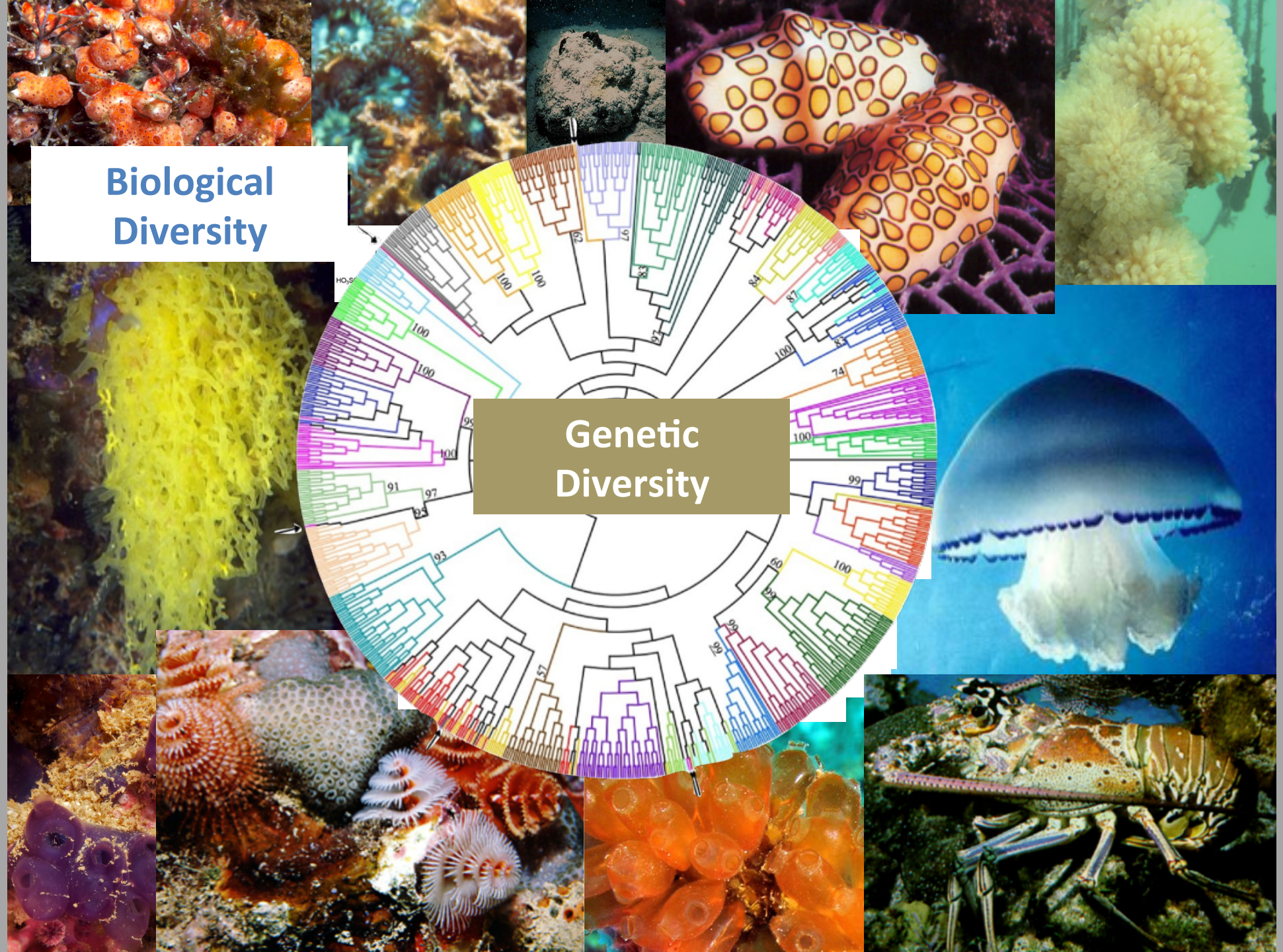
Biological Diversity



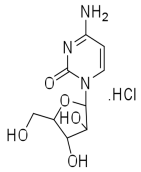
Biodiversity

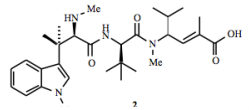
http://comlmaps.org/oceanlifemap/past-present-future

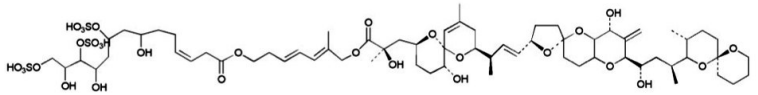

Genetic
Diversity

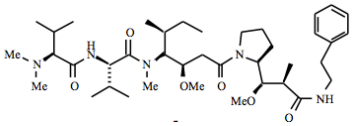

Biological Diversity
Genetic Diversity

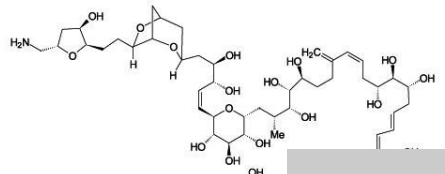
Chemical Diversity

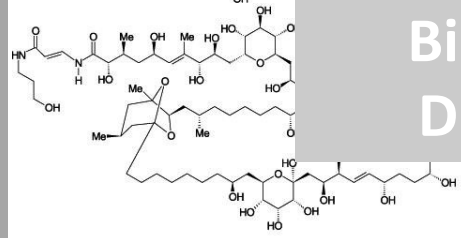


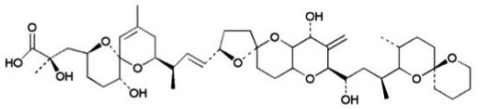


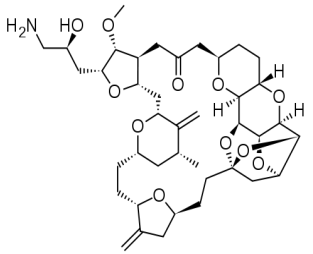




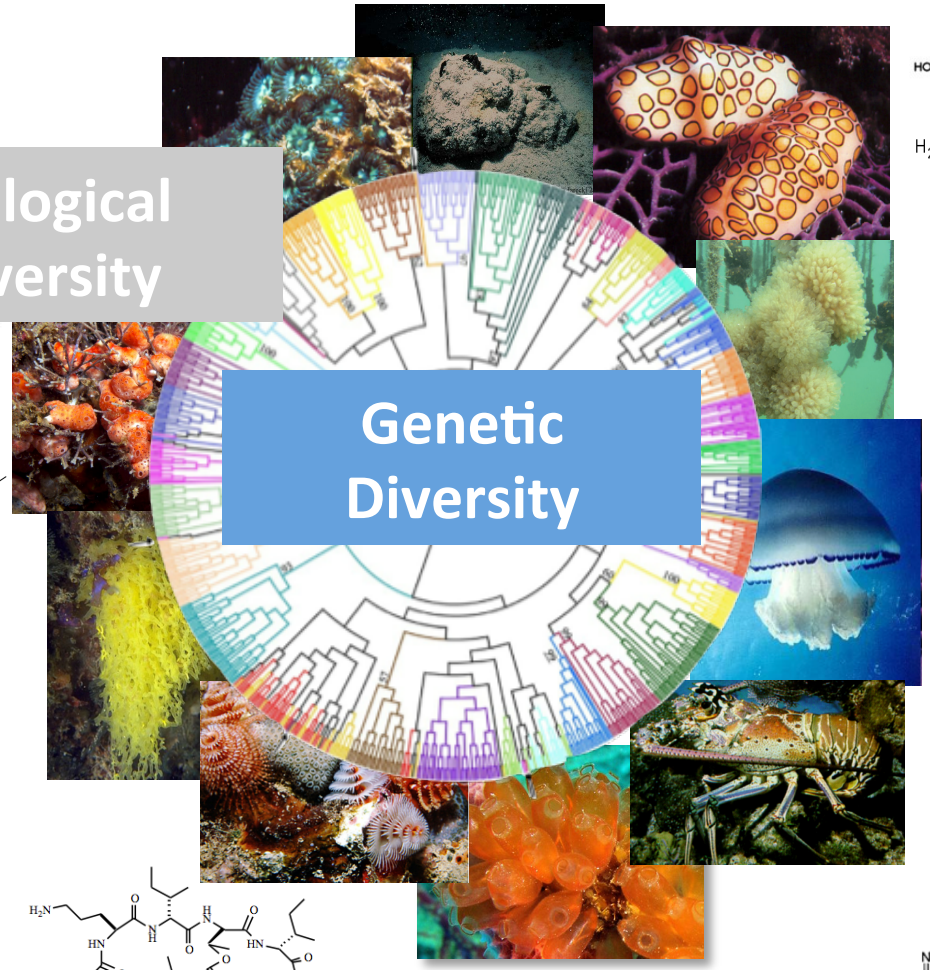




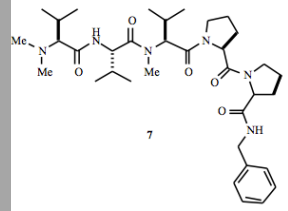


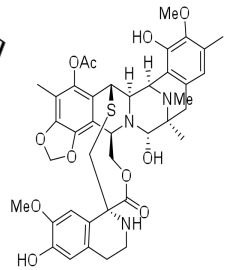


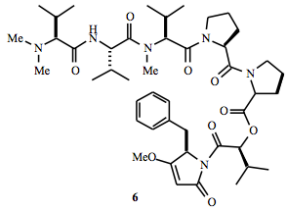
Biological Diversity

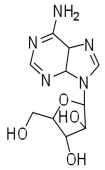


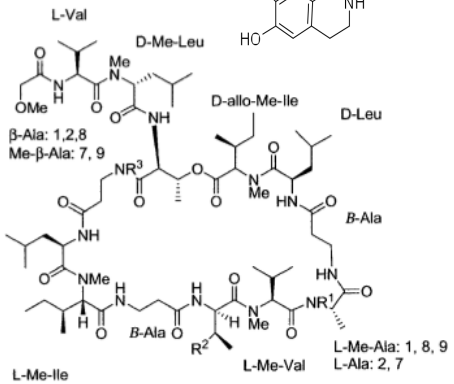
Genetic Diversity

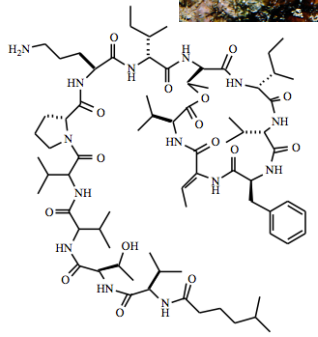


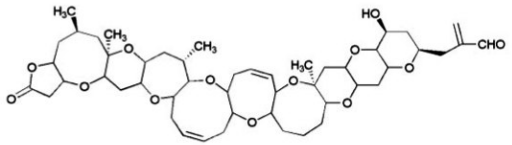


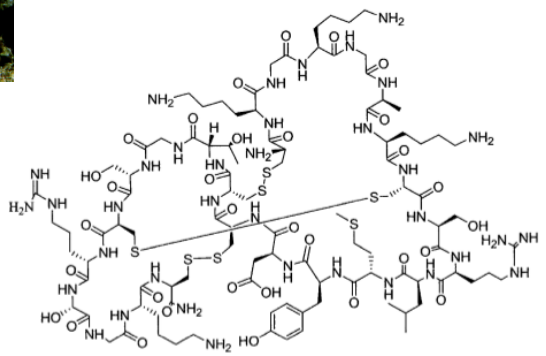


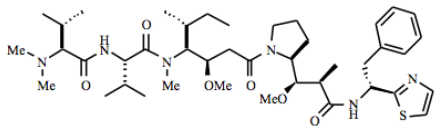




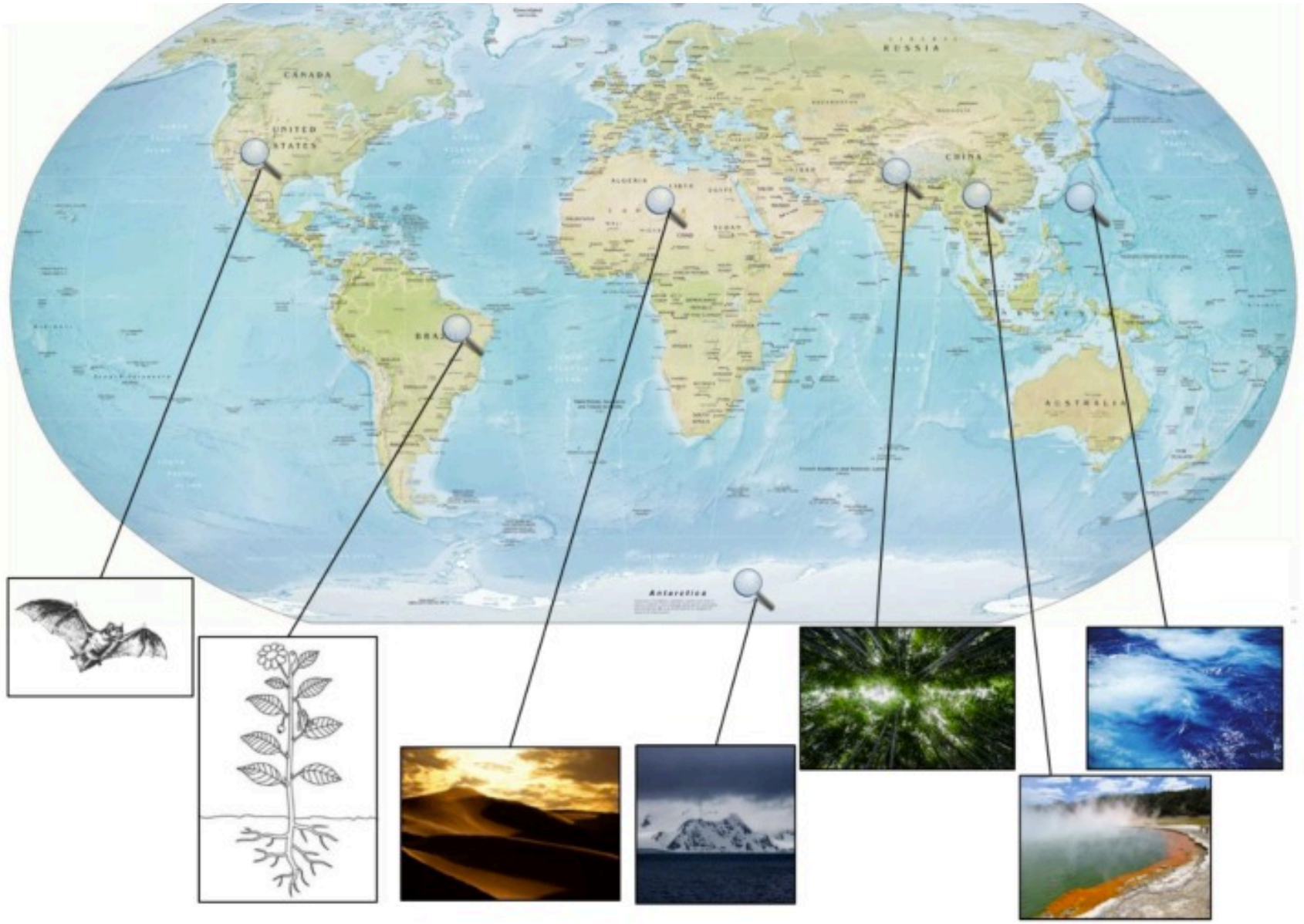




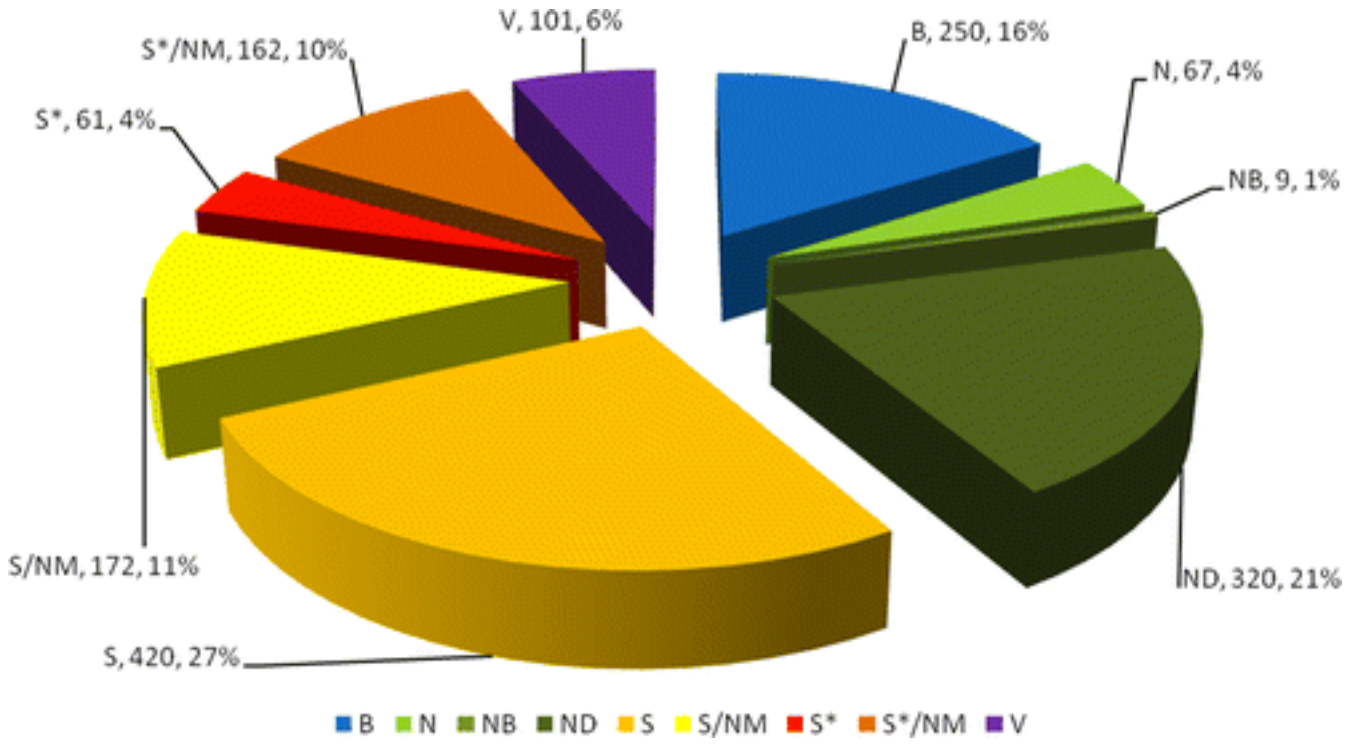




Underexploited habitats of actinobacteria attracted more attention for microbial natural product discovery. Currently, oceans, deserts, mountains and Antarctica ranges together with hot springs and endophytes and symbionts are focuses of the search for new bioactive compounds.

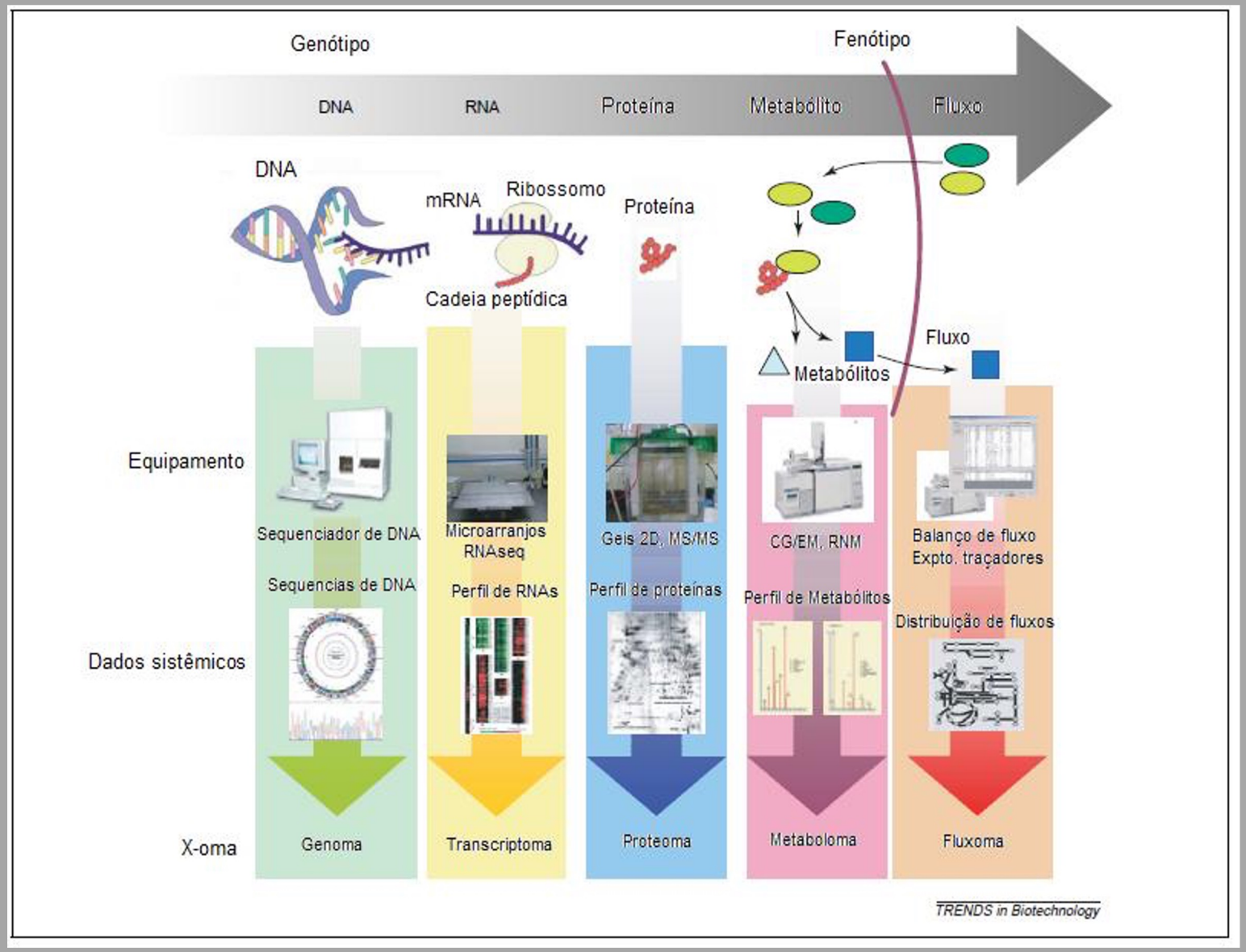


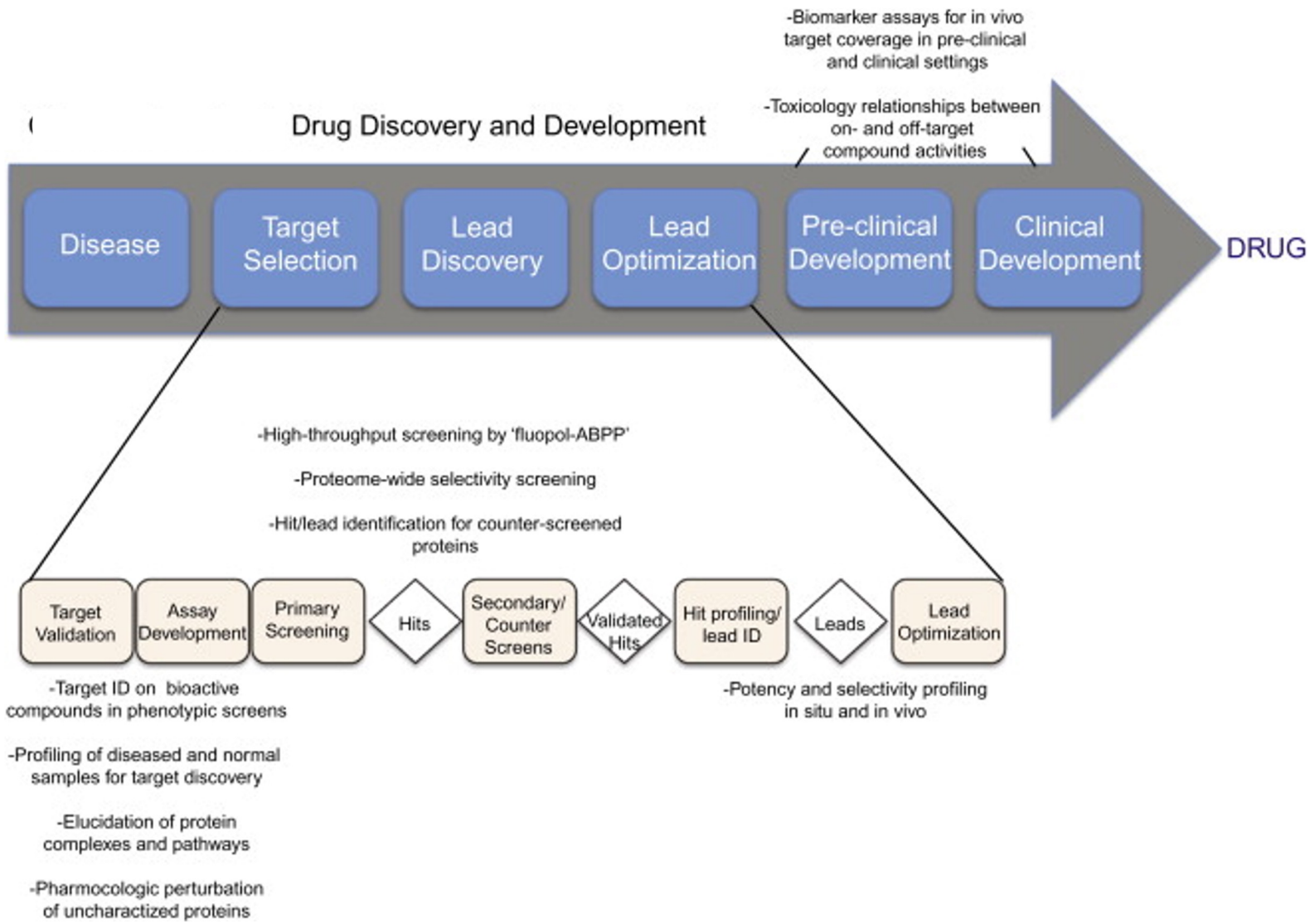
All new approved drugs 1981–2014; n = 1562



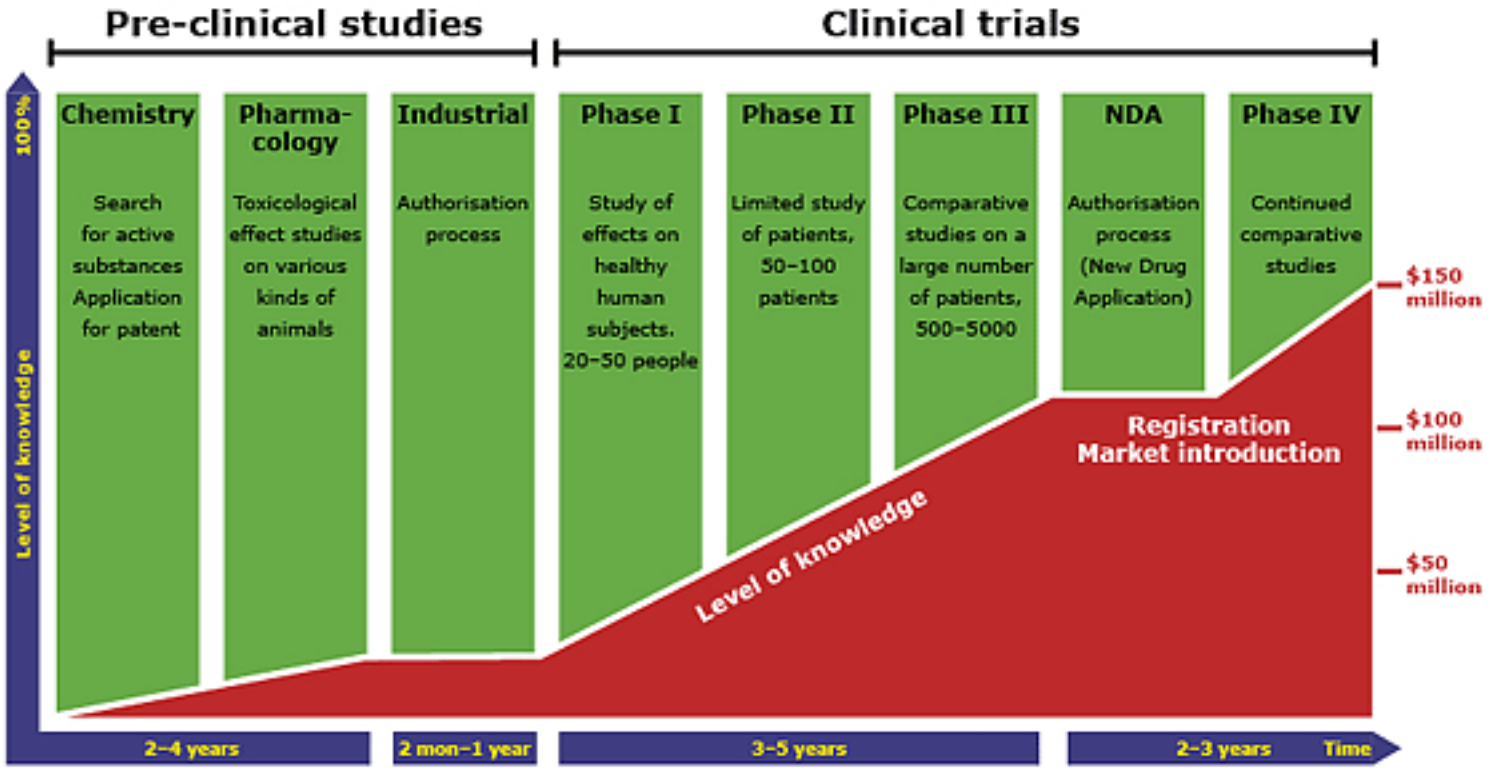
B= biological macromolecule; N= unaltered natural product; NB= botanical drug; ND= natural product derivative; S= synthetic drug; S*= synthetic drug (NP pharmaphore); V= vaccine; /NM= mimic of natural product

Omics in Biotecnology

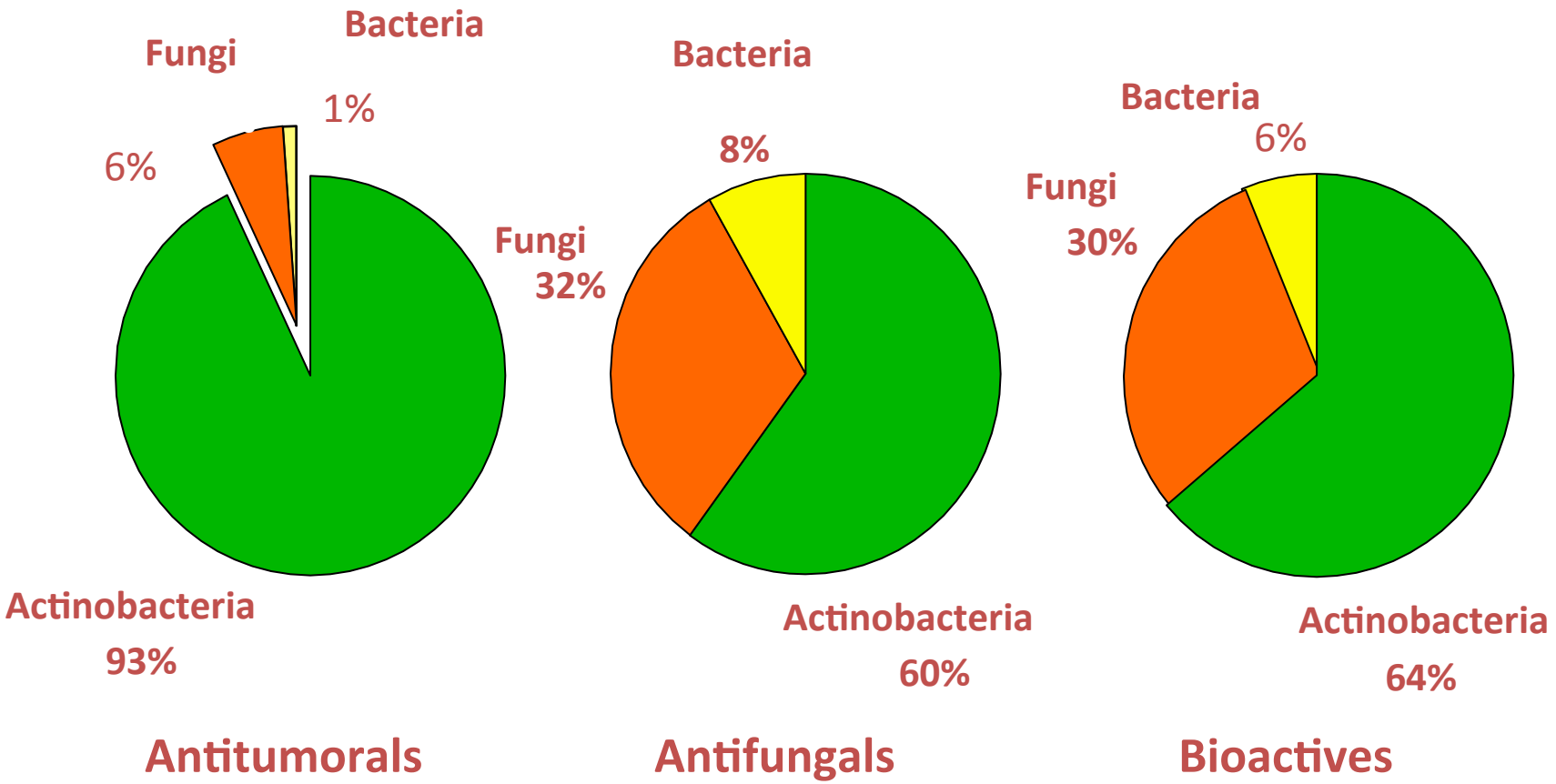



Drug Discovery and Development
-Biomarker assays for in vivo target coverage in pre-clinical and clinical settings
-Toxicology relationships between on- and off-target compound activities
Disease
Target Selection
Lead Discovery
Lead Optimization
Pre-clinical Development
Clinical Development
DRUG
-High-throughput screening by 'fluopol-ABPP'
-Proteome-wide selectivity screening
-Hit/lead identification for counter-screened proteins
Target Validation
Assay Development
Primary Screening
Hits
Secondary/ Counter Screens
Validated Hits
Hit profiling/ lead ID
Leads
Lead Optimization
-Target ID on bioactive compounds in phenotypic screens
-Profiling of diseased and normal samples for target discovery
-Elucidation of protein complexes and pathways
-Pharmocologic perturbation of uncharactized proteins
-Potency and selectivity profiling in situ and in vivo

Desenvolvimento de Novas Drogas



MICROORGANISMS and BIOACTIVE COMPOUNDS



What are antibiotics?

- Any compund able to kill a target cell
- Secondary metabolites synthesized by some microorganisms
- Biological activity

Who are the main producers

- Bacteria

  Gram positive *Streptomyces*
- Fungi
- Other bacteria

Biological functions of antibiotics?

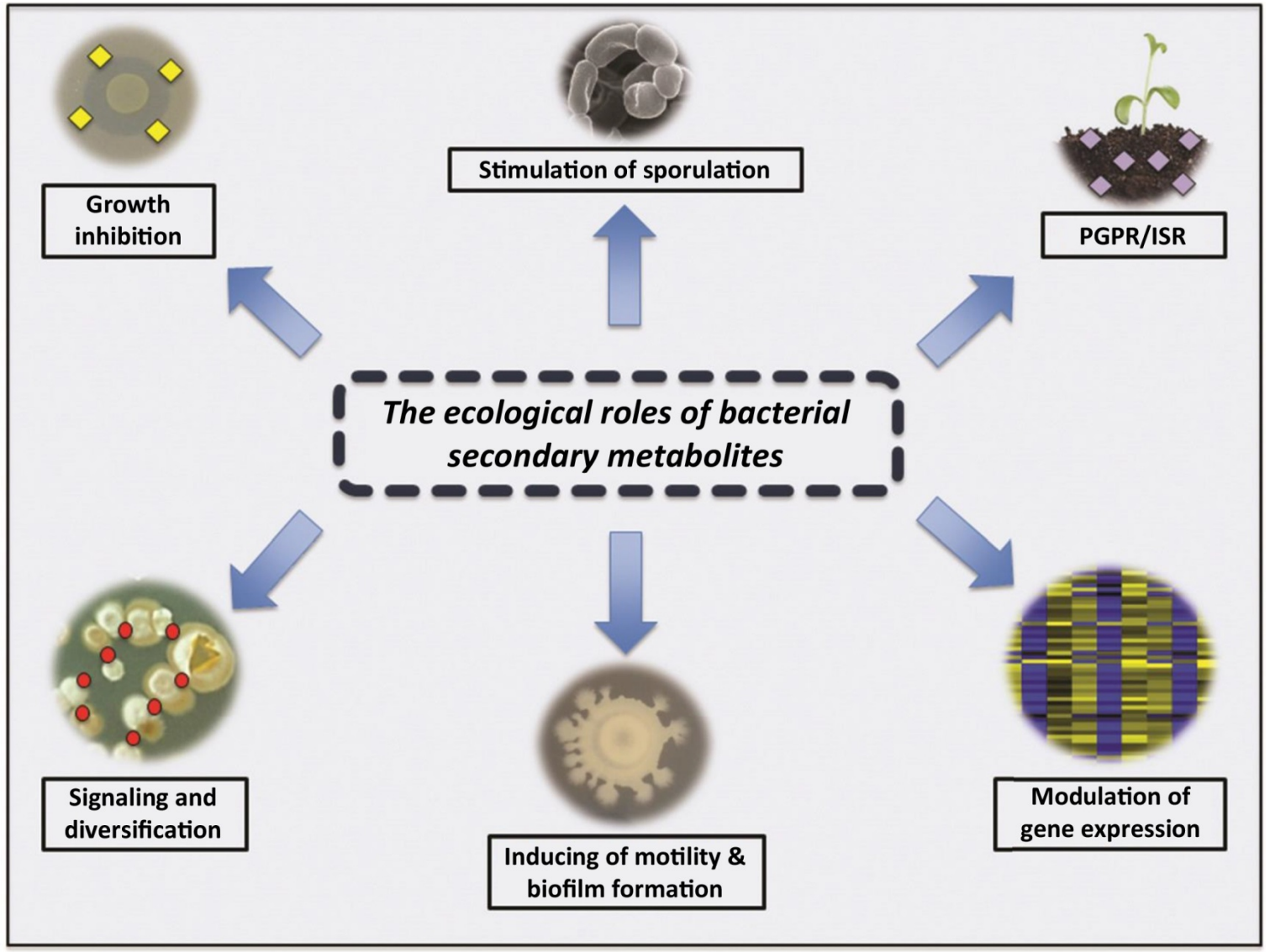
- In the producer:

  Activators of morphological differentiation, UV protector, communication

- In the target microorganism:

  Toxicity

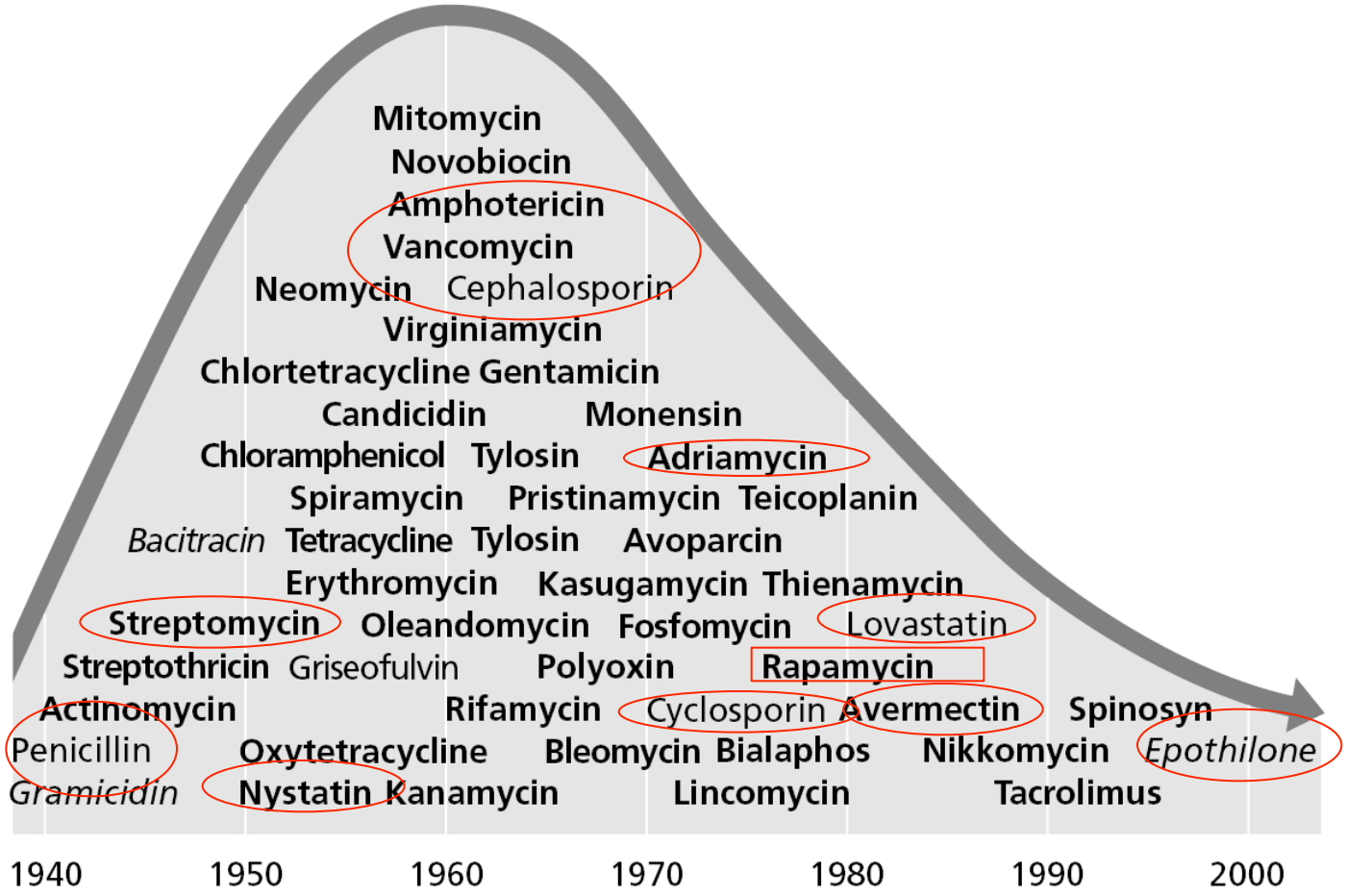
Different Ecological Roles of Bacterial Secondary Metabolites in Nature



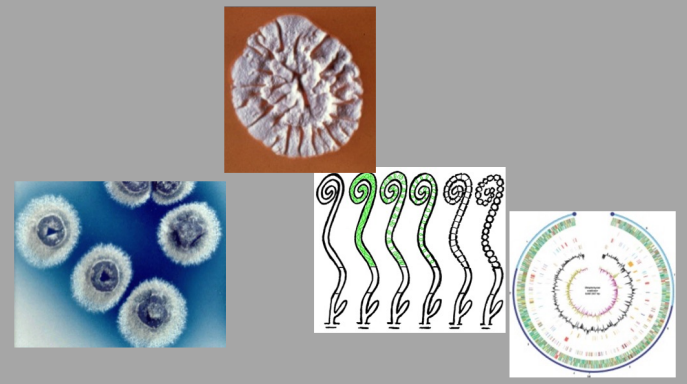
Cell PRESS

Trends in Microbiology

Diminishing returns in finding natural products: Genetics to the rescue?



***Streptomyces* spp.**



Gram-positive bacteria

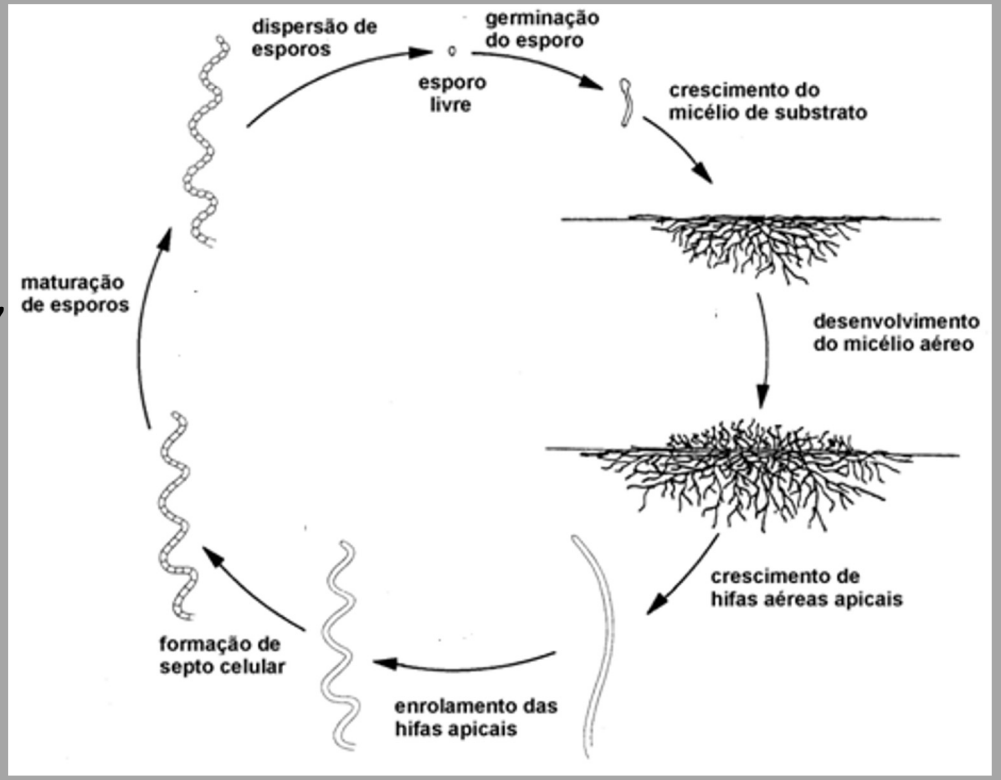
Habitats: soil, marine environments, endophytic

Linear chromosome of ≈ 8Mb

High GC content (69-74%)

Biomolecules of interest for medicine, veterinary and agriculture

Lyfe cycle



BIOACTIVE COMPOUNDS SYNTHESIZED BY ACTINOMYCETES

ANTIBACTERIALS

Erythromycin
Tetracycline
Gentamicin

ANTIFUNGALS

Amphotericin B
Nystatin

ANTIPARASITICS

Avermectins

ANTITUMORALS

Doxorubicin
Mitramycin
Bleomycin

IMUNOSUPRESSANTS

Rapamycin
FK506

INSETICIDES

Espinosin

HERBICIDES

Bialaphos

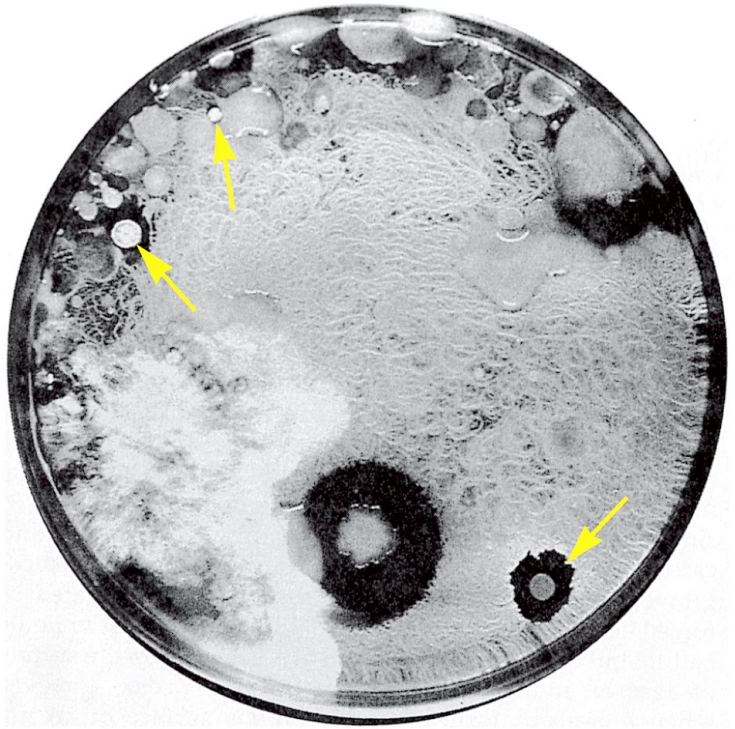


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Figure 12-77a Brock Biology of Microorganisms 11/e
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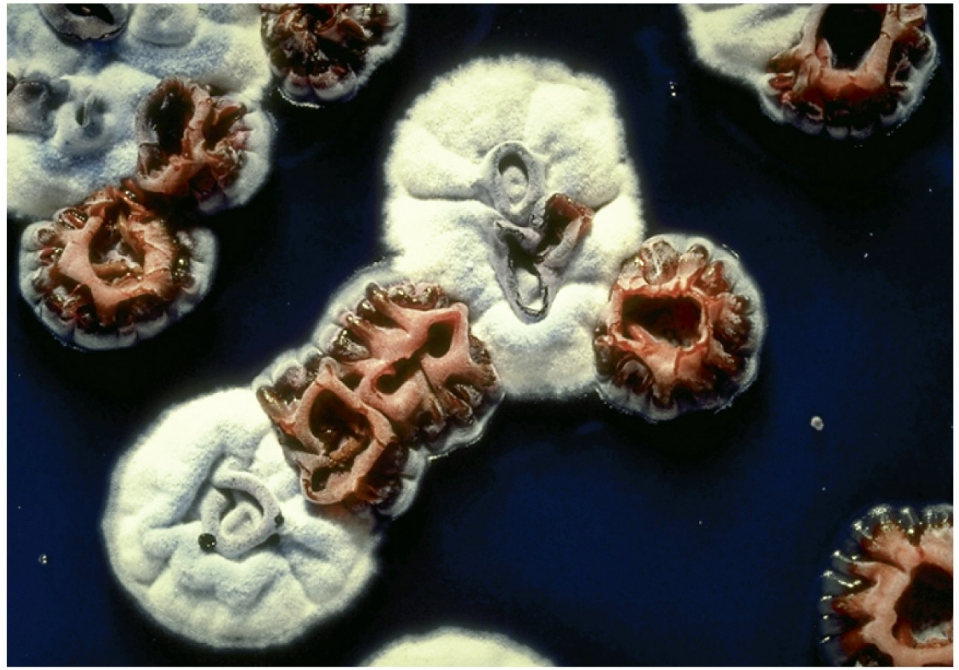

David A. Hopwood

Figure 12-77b Brock Biology of Microorganisms 11/e
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Antibiotics classifications

Origin: natural, semisynthetic, syntethic

Chemical structures (11 groups)

Biological Activity

Spectrum of Activity

Biological Target

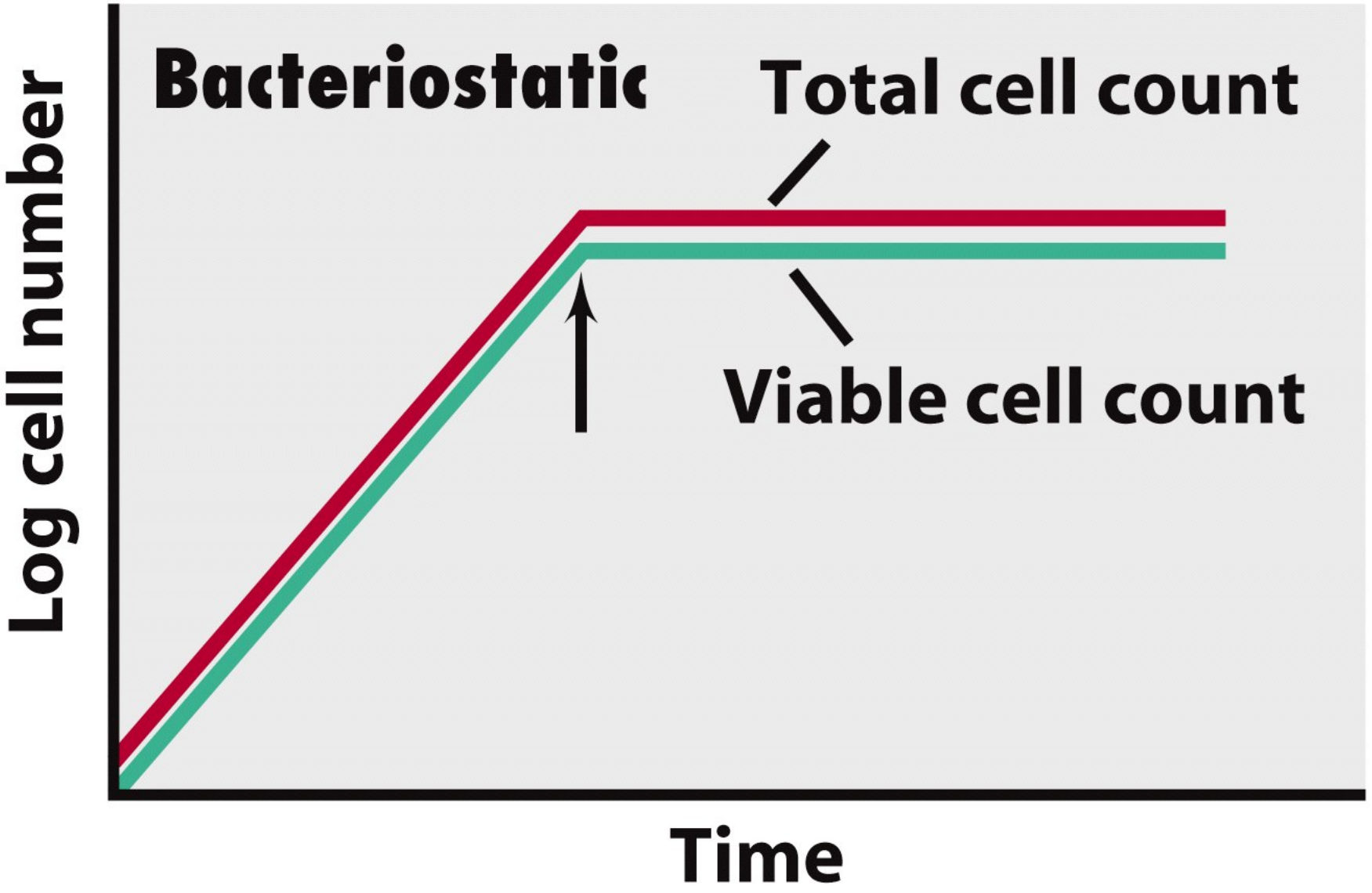


Figure 20-9a Brock Biology of Microorganisms 11/e

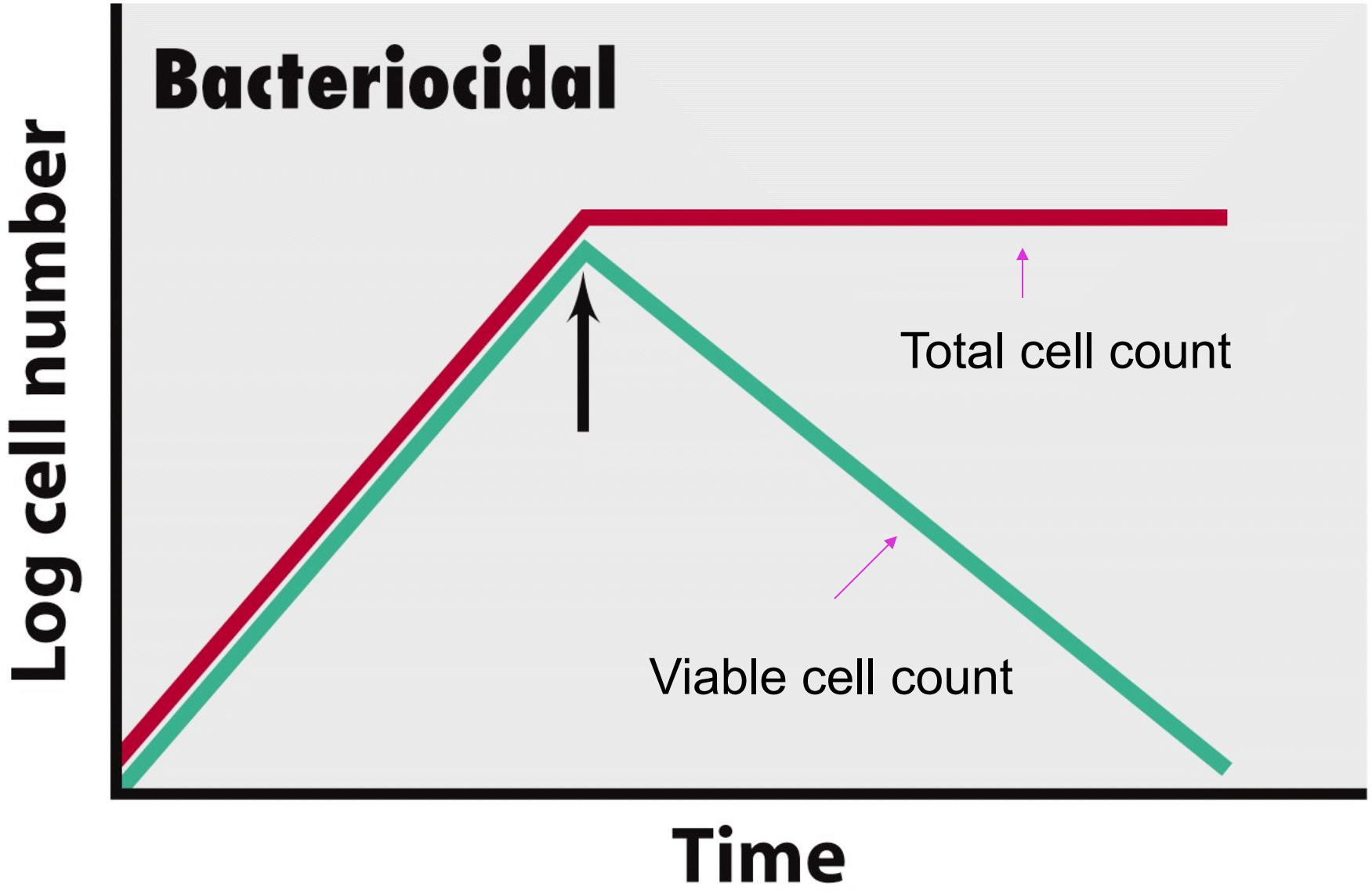


Figure 20-9b Brock Biology of Microorganisms 11/e

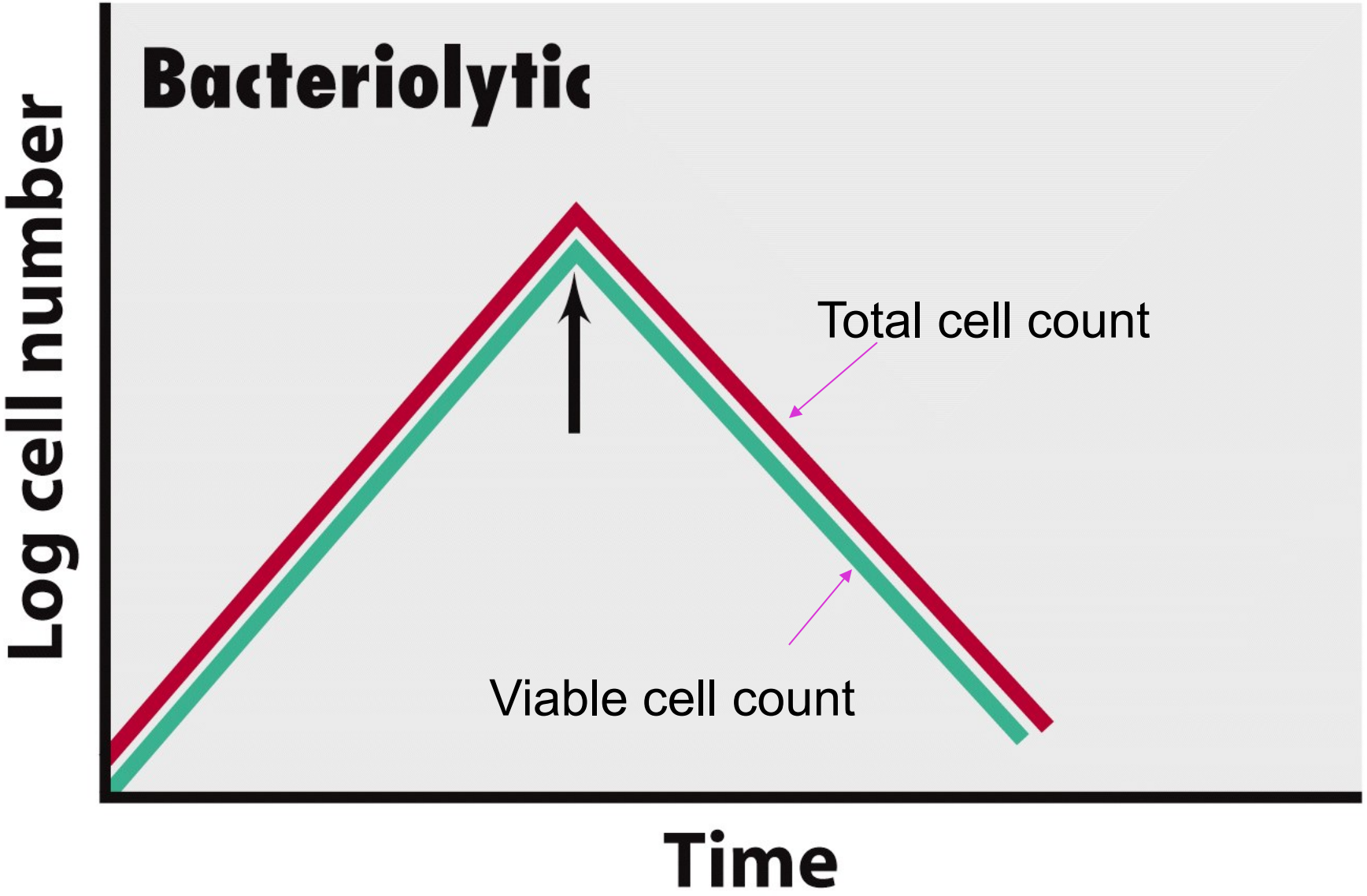
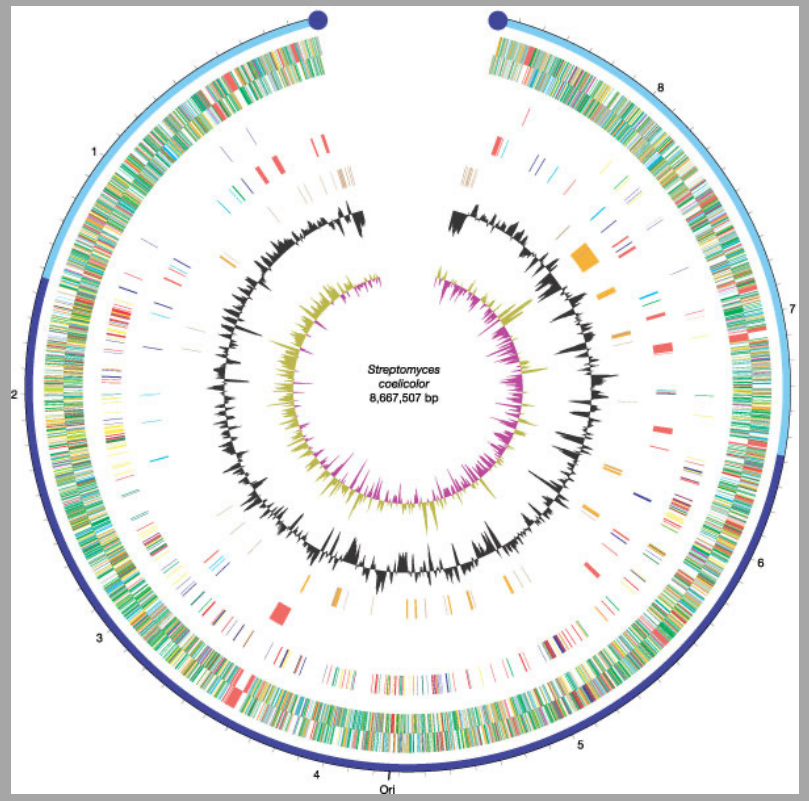


Figure 20-9c Brock Biology of Microorganisms 11/e

APPROACHES FOR SEARCHING NEW BIOACTIVE COMPOUNDS

- **COMBINATORIAL CHEMISTRY**
  - Libraries of compounds from chemical synthesis
- **NEW UNEXPLORED ECOLOGICAL NICHES**
  - Marine organisms
  - Amazonas biodiversity
  - Endophytic organisms
- **COMBINATORIAL BIOSYNTHESIS**
  - Combination of genes from biosynthetic pathways of bioactiove compounds

Descoberta de novos “clusters” biossintéticos por projetos genoma

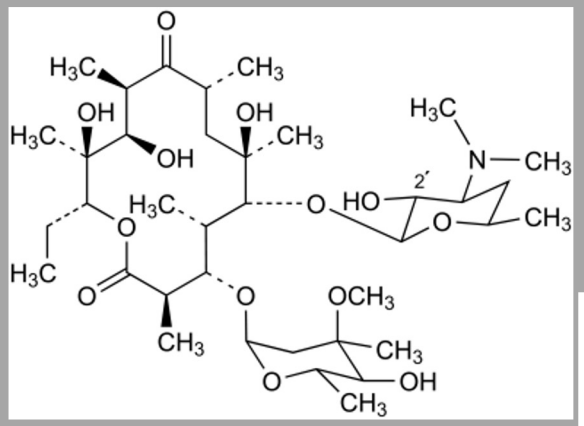


Nature 417; 141-147 (2002) Bentley *et al.*

General features of the *Streptomyces* genome

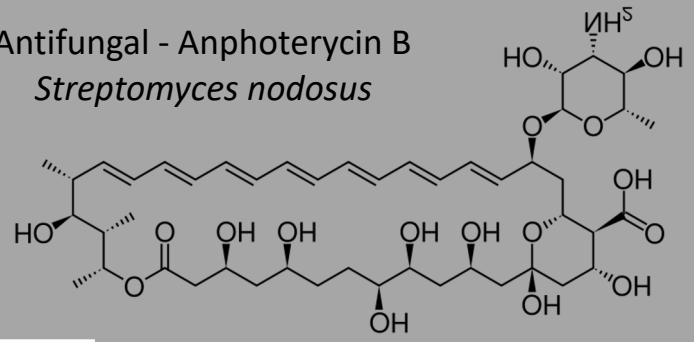
Component of chromosome Property	
Total size	8 667 507 bp
Terminal inverted repeat	21 653 bp
G+C content	72.12%
Coding sequences	7825
Pseudogenes	55
Coding density	88.9%
Average gene length	991 bp
Ribosomal RNAs	6x(16S-23S-5S)
Transfer RNAs	63
Other stable RNAs	3

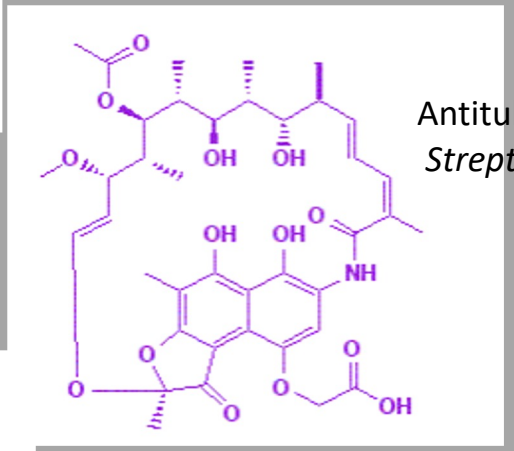
Polyketide compounds



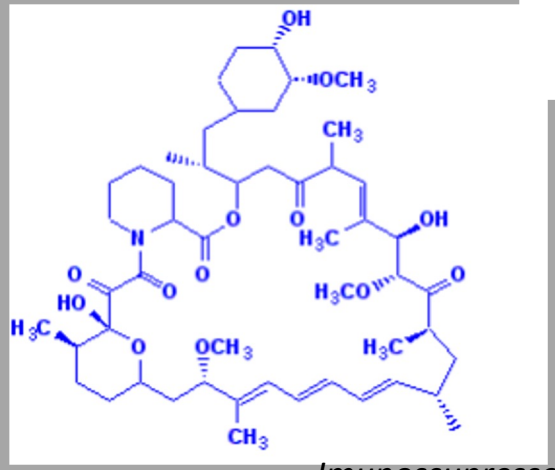
Antibacterial- erythromycin
Streptomyces erythreus

Antifungal - Anphoterycin B
Streptomyces nodosus

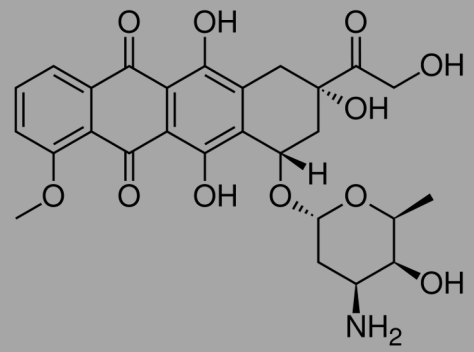




Antituberculose- Rifamycin
Streptomyces mediterranei

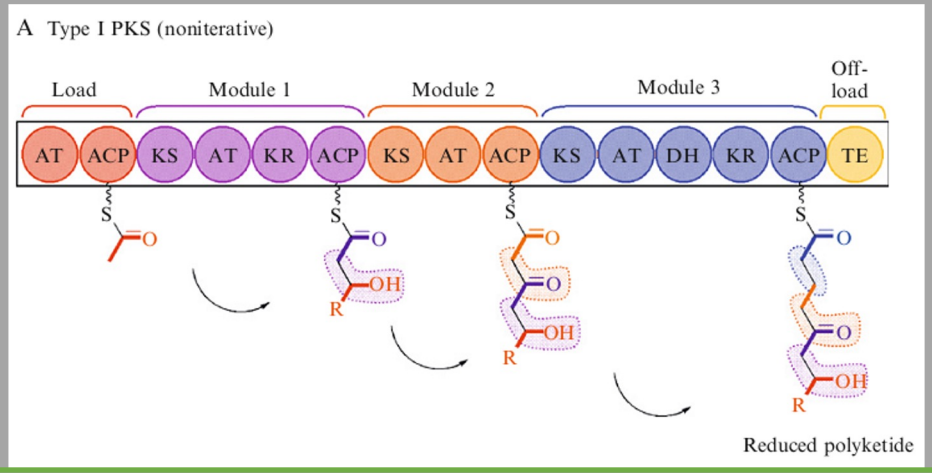


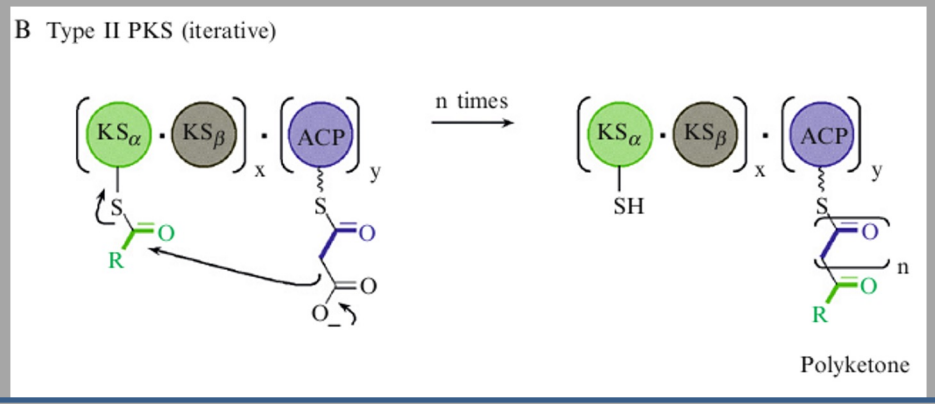
Imunossupressor – Rapamycin Streptomyces hygroscopicus



Anticancer – Doxorubicine
Streptomyces peucetius

PKS I



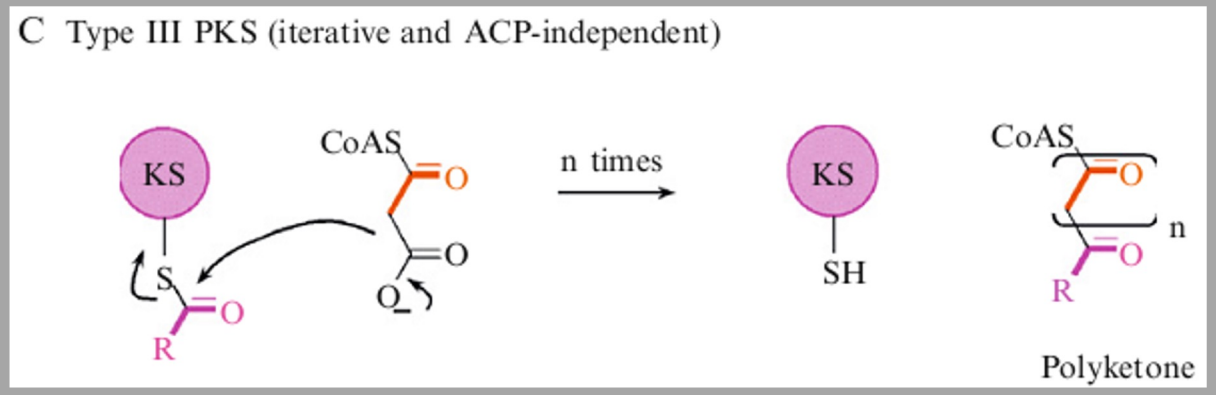


PKS II

ANTITUMORAL

ANTIBIOTICS

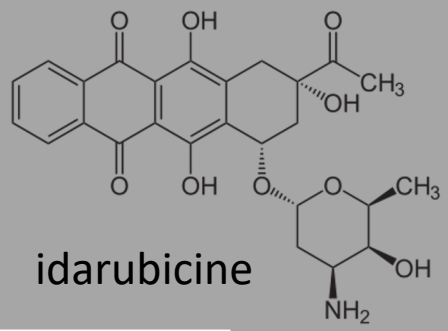
PKS III



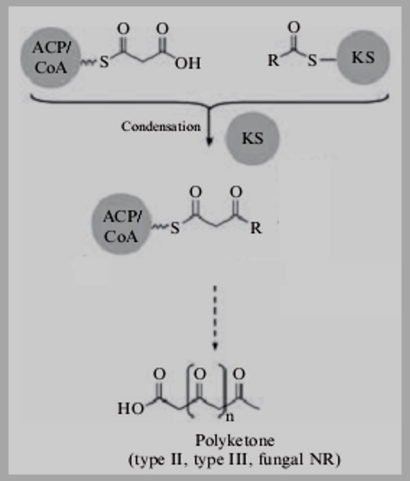
Anthracyclines - Streptomyces

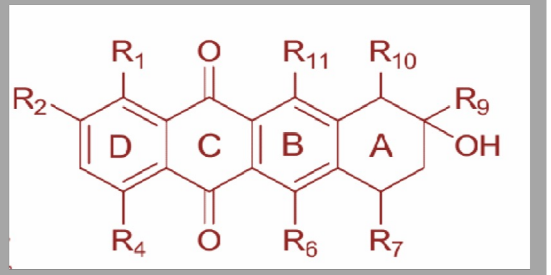
Basic structure

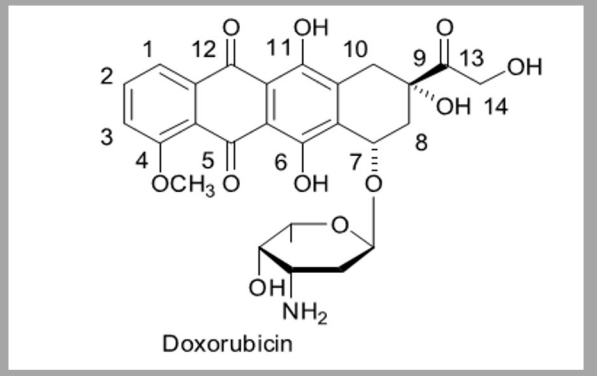
Compounds synthesized via PKS II



idarubicine

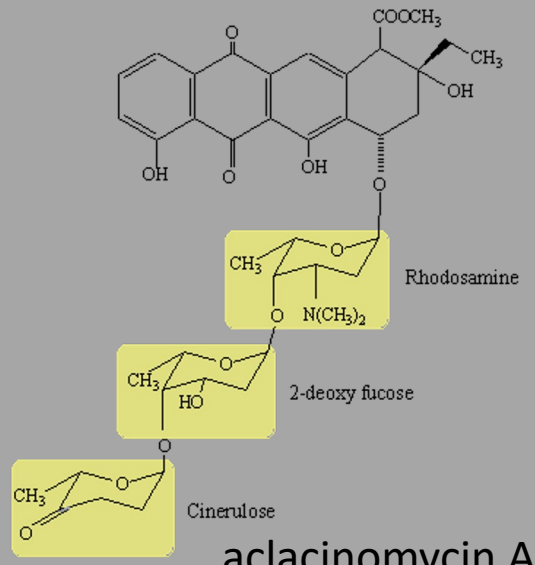






Doxorubicin

Posição	Substituinte
R1	H, OH glicosídeo
R2	H, OH glicosídeo
R4	OH, OCH_3
R6	H, OH
R7	H, OH glicosídeo
R9	CH_3, CH_2 CH_3, $COCH_3$, $COCH_2OH$, $CHOHCH_3$, $CHOHCH_2OH$, CH_2COCH_3
R10	H, OH, $COOCH_3$, glicosídeo
R11	H, OH



aclacinomycin A

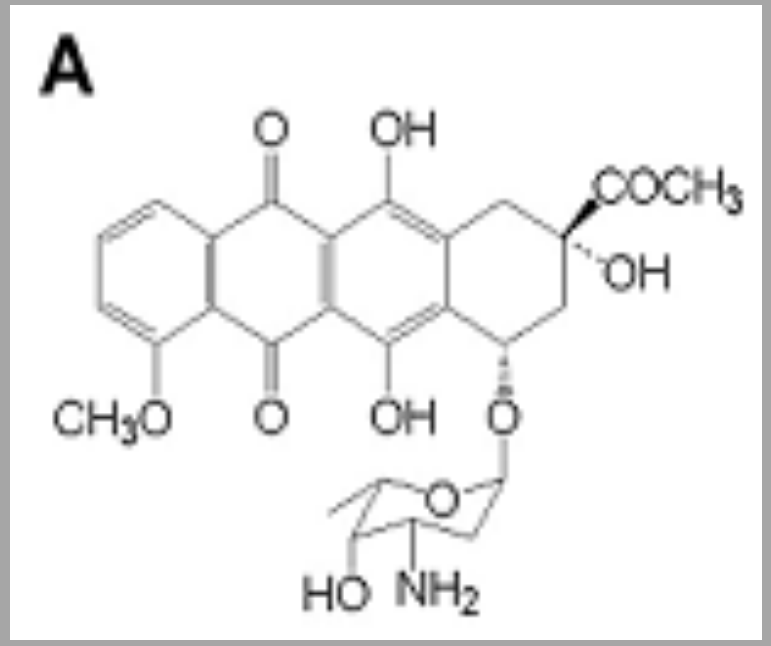
Anthracyclines

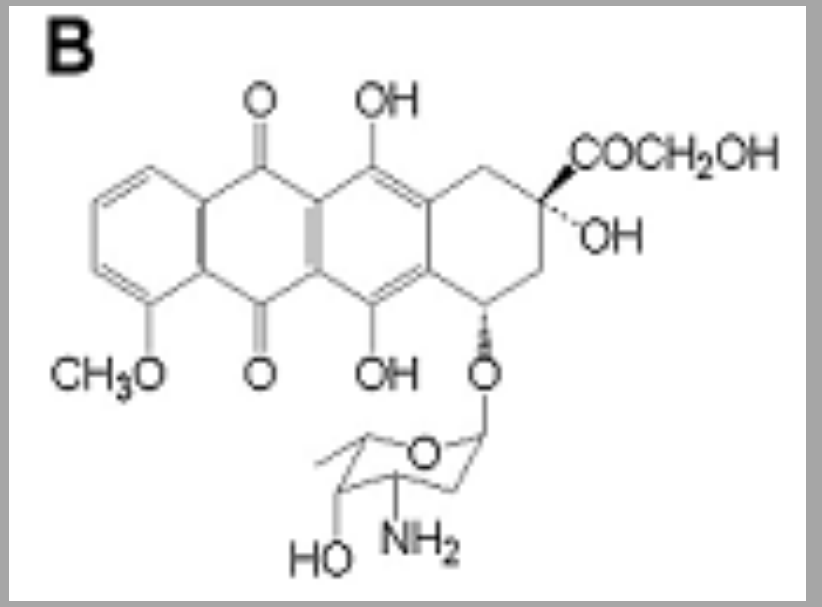
1950	*Streptomyces purpurascens*	Rhodomycin
1958	Farmitalia - *S. peucetius*	Daunomycin
1962	Rhône Poulanc	Rubidomycin
1963	Anticancer activity	
1965/ 70	compound renamed citotoxic activity against solid and ascites tumors	Daunorubicin
1969	Daunorubicin analog adriamycin *S. peucetius* subs. *Caesius*	Doxorubicin
1974	APPROVED by FDA	

MAIN PROBLEMS: **toxicity** **cardiotoxicity**

MDR (multiple-drug-resistance) phenotype

Structures of the anthracyclines:

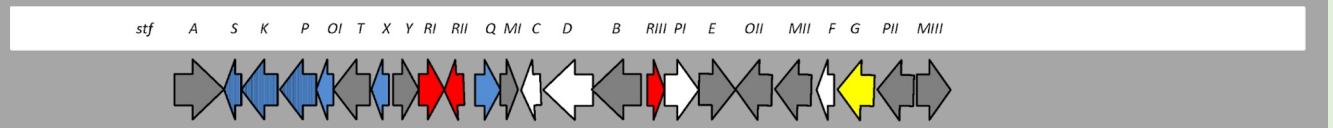


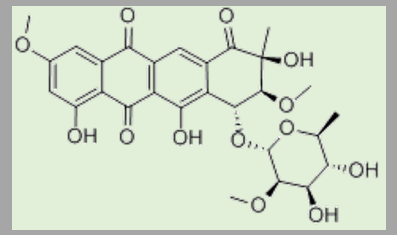


(A) daunorubicin (daunomycin). (B) doxorubicin (adriamycin)

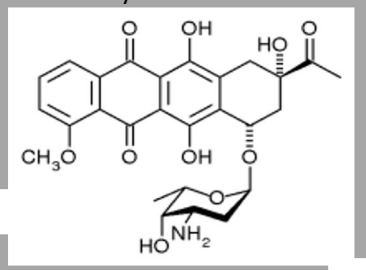
Anthracycline biosynthetic clusters

Estefimycin (stf) - *Streptomyces steffisburgensis NRRL 3193 [*

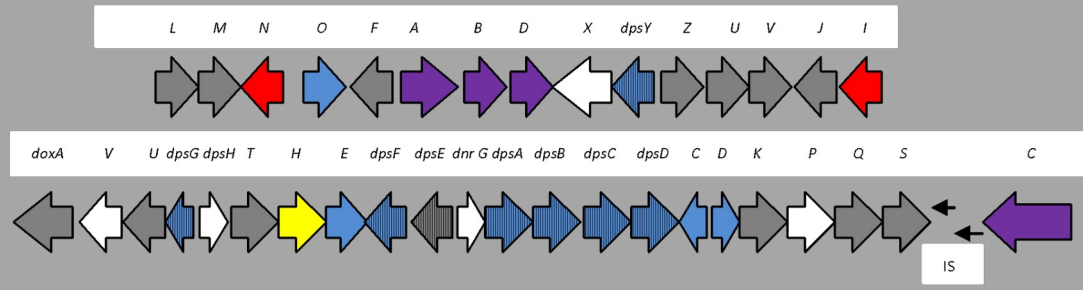


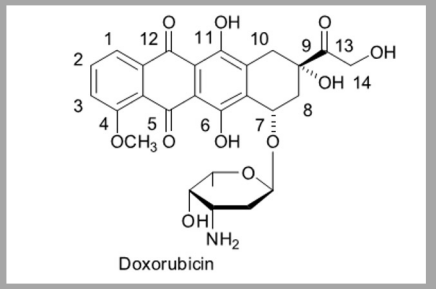


estefiimycin



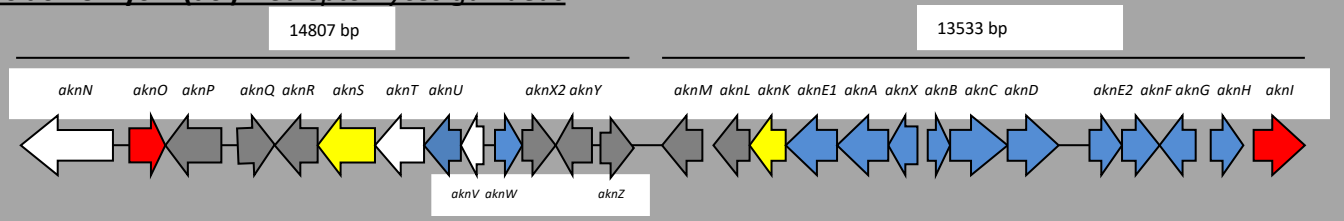
Doxo/daunorubicin (stf) - *Streptomyces peucetius*

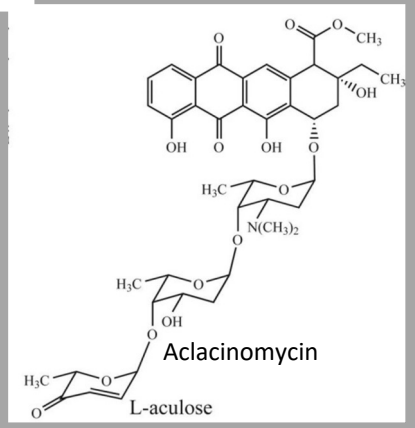




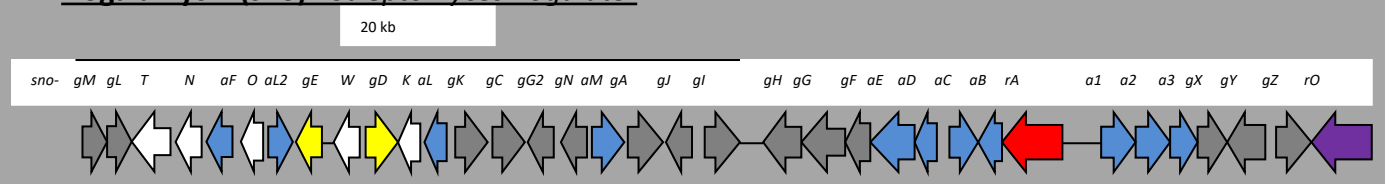
Daunorubicin

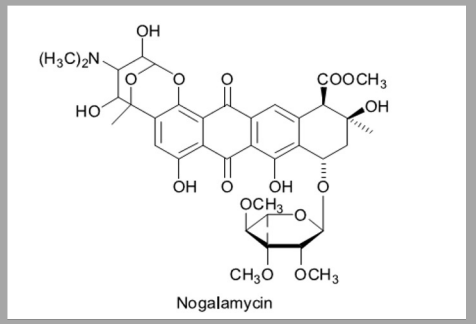
Aclacinomycin (acl) – *Streptomyces galilaeus*





Nogalamycin (sno) - *Streptomyces nogalater*

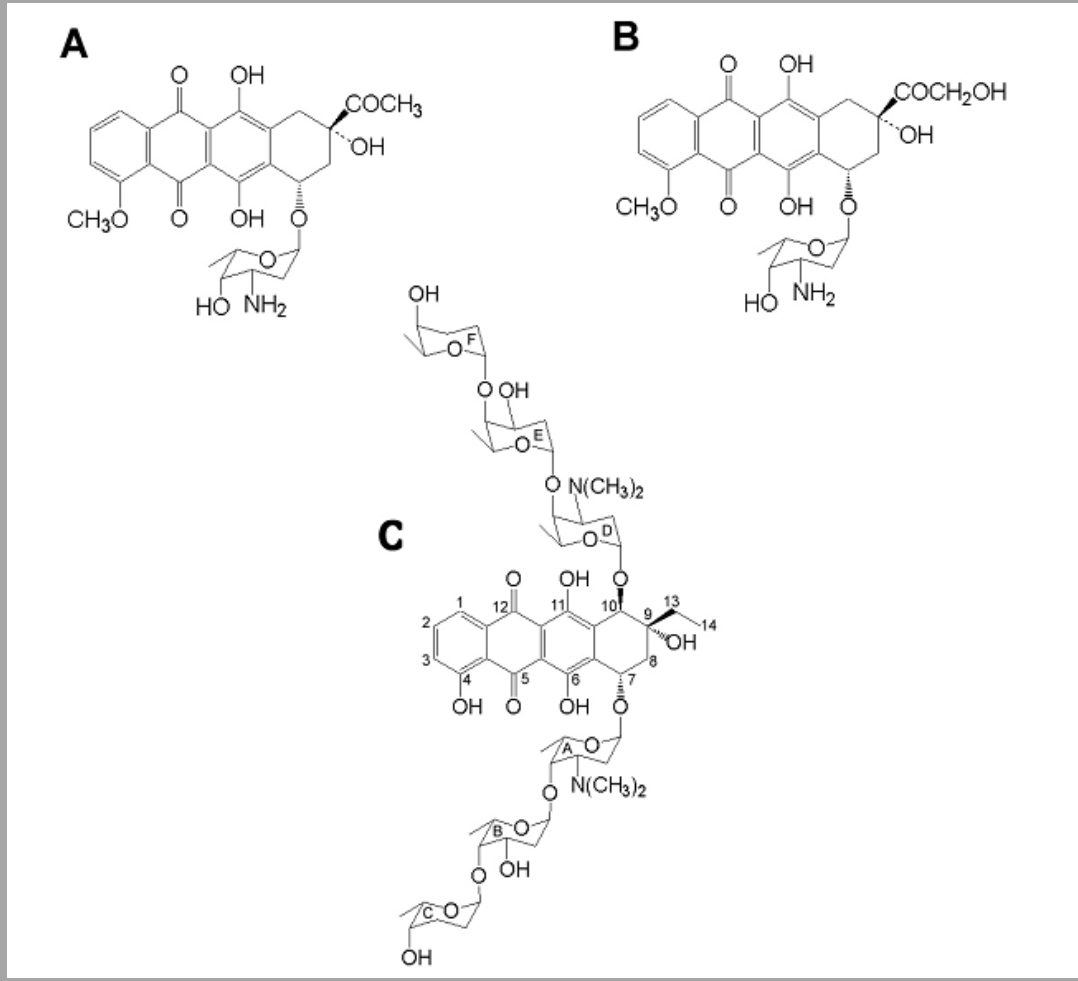




Sugar biosythesis/modification | PKS minimal | Aglycone | Unknown

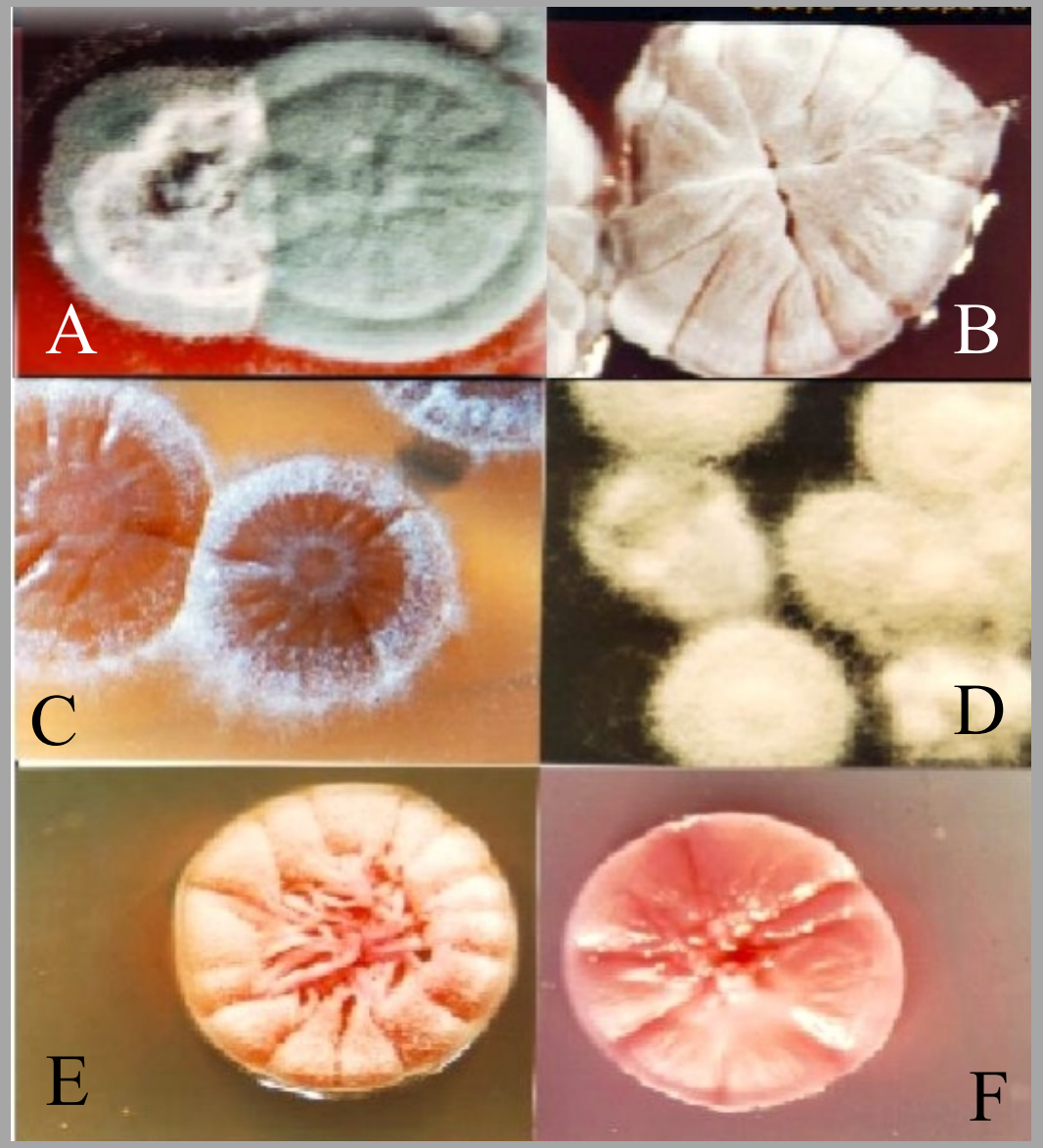
Glycosiltransferase | Regulator | Resistence

Structures of anthracyclines:



(A) daunorubicin (daunomycin). (B) doxorubicin (adriamycin), and (C) Csmomycin D purified from *Streptomyces olindensis* ICB20.

Streptomyces olindensis



A. Wild Type

B. SOV 1

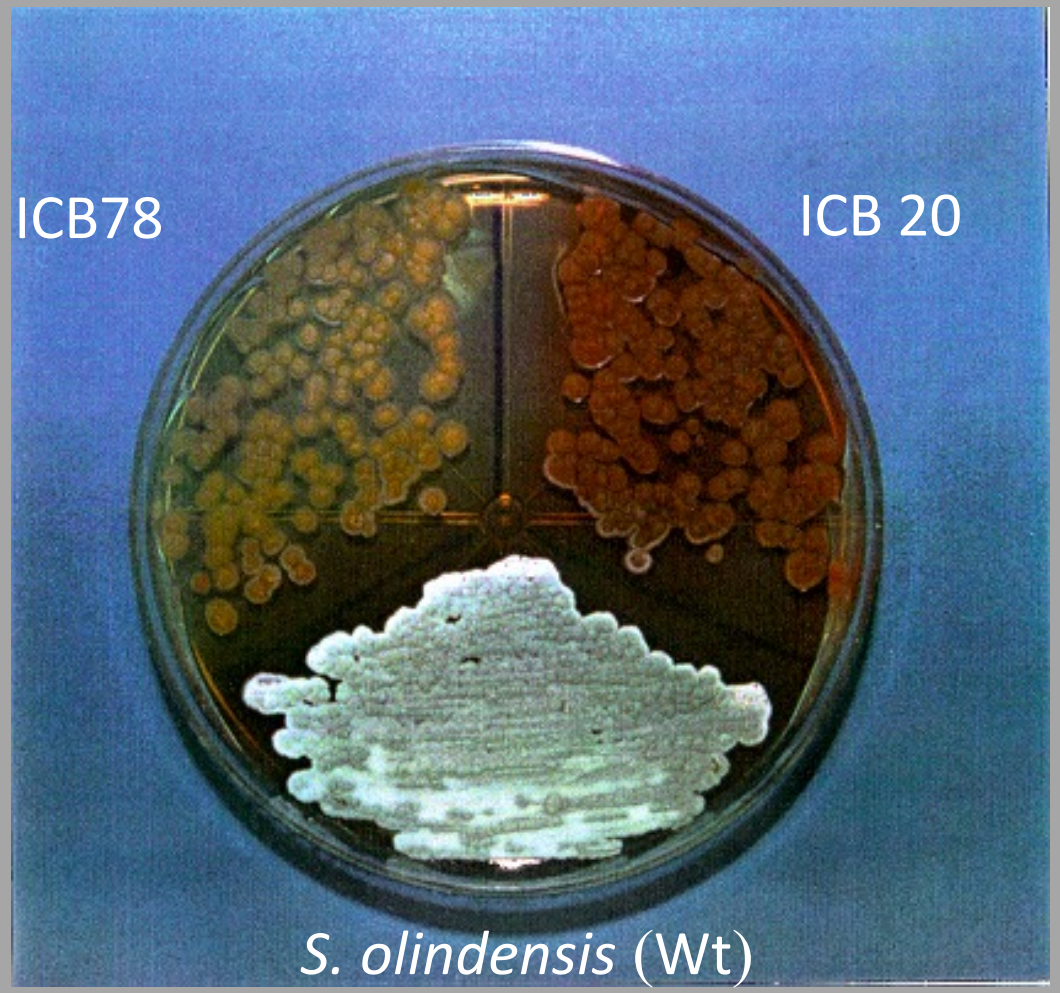
C. SOV 2

D. SOV 6

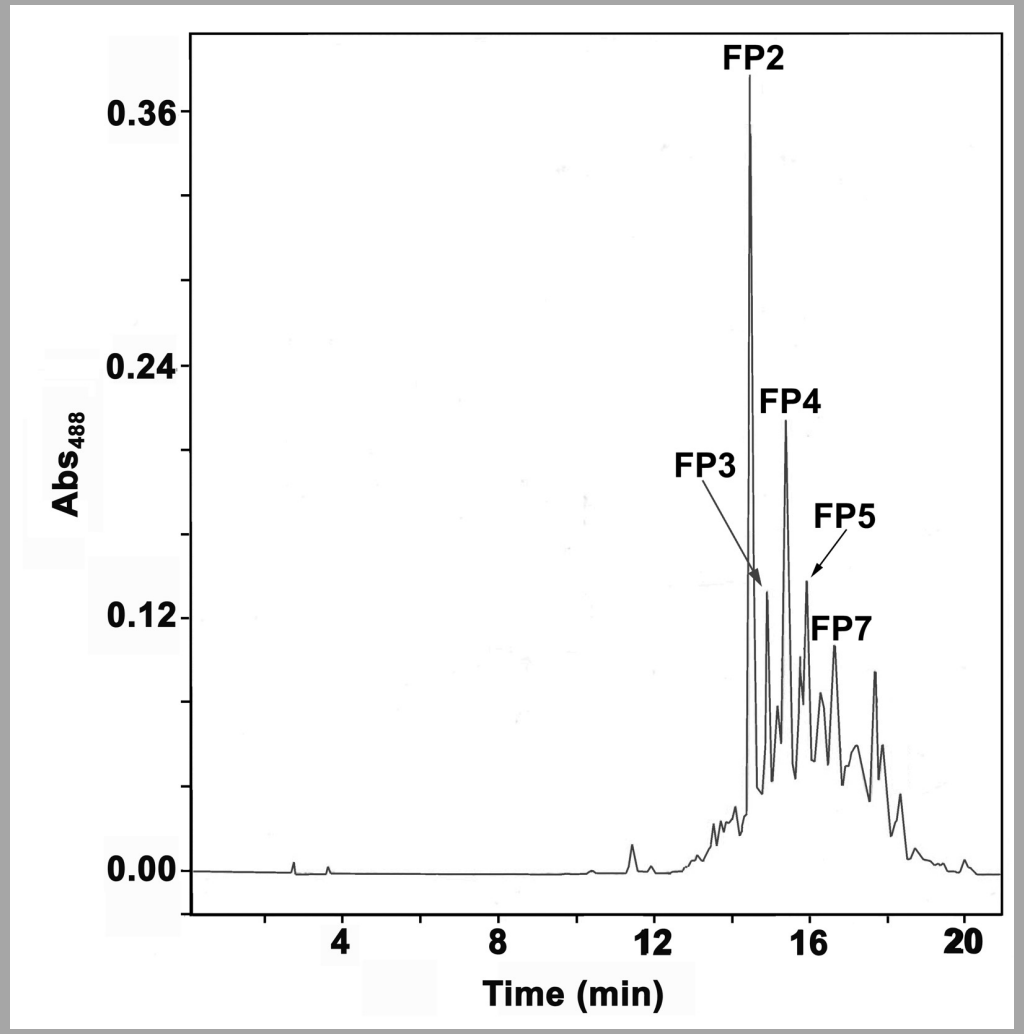
E. SOV 16

F. SOV 30

Streptomyces olindensis DAUFPE 5622



HPLC elution profile for supernatants from *S. olindensis* ICB20



concentration (mg/L)

FP2: 37.6

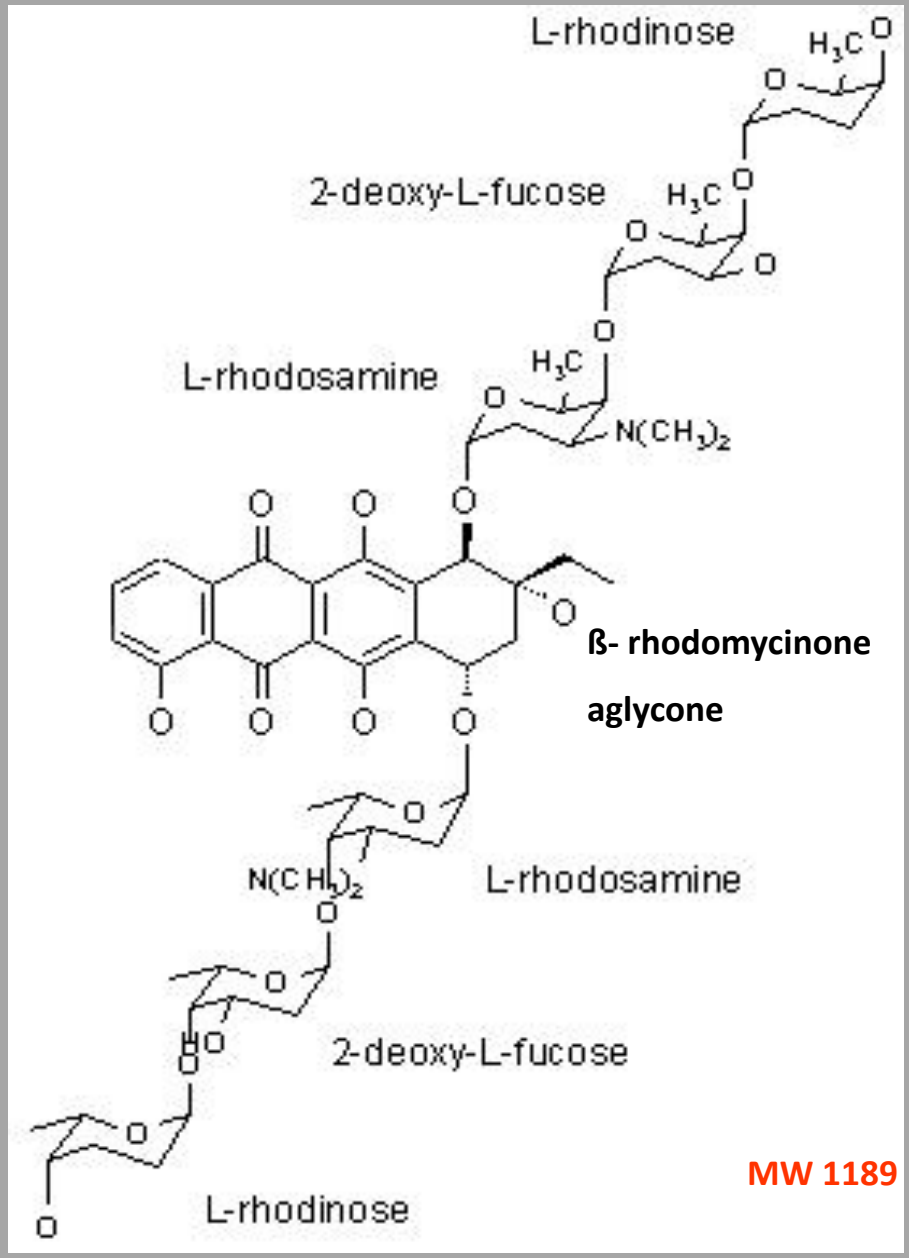
FP3: 8.1

FP4: 11.5

FP5: 11.6

FP7: 11.8

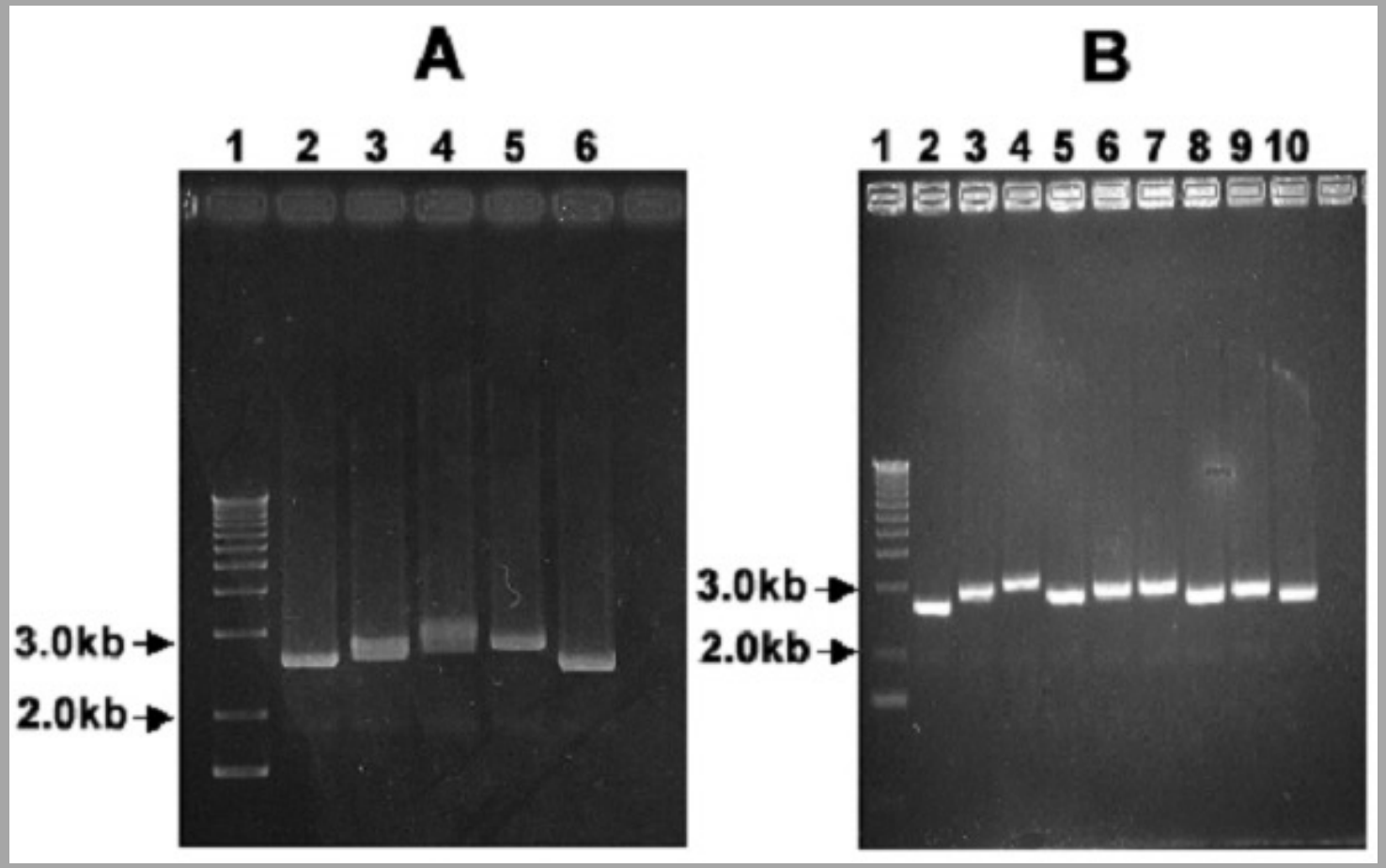
Seven major fractions: FP1-FP7



Cosmomycin D purified from *Streptomyces olindensis* ICB20.

Anthracycline mechanisms of action:

1. Non covalent intercalation with DNA (L)
2. Formation of DNA aducts and “cross- linking”
3. Topoisomerase II inhibtion
4. Inhibition of DNA and RNA synthesis
5. Free radicals formation
6. Apotosis induction



Gel mobility shift assay comparing migration of complexes.

(A) 1. Standard DNA ladder. 2. Control pUC18. 3. DAU (10 ng/ml). 4. DOX (10 ng/ml). 5. FUR (10 ng/ml). 6. Control pUC18**. (B)** 1. Standard DNA ladder. 2. Control pUC18. 3.-9. FP1-FP7 (all at 5 ng/ml). 10. Control pUC18.

Biotechnology Letters (Furlan *et al.*, 2002)