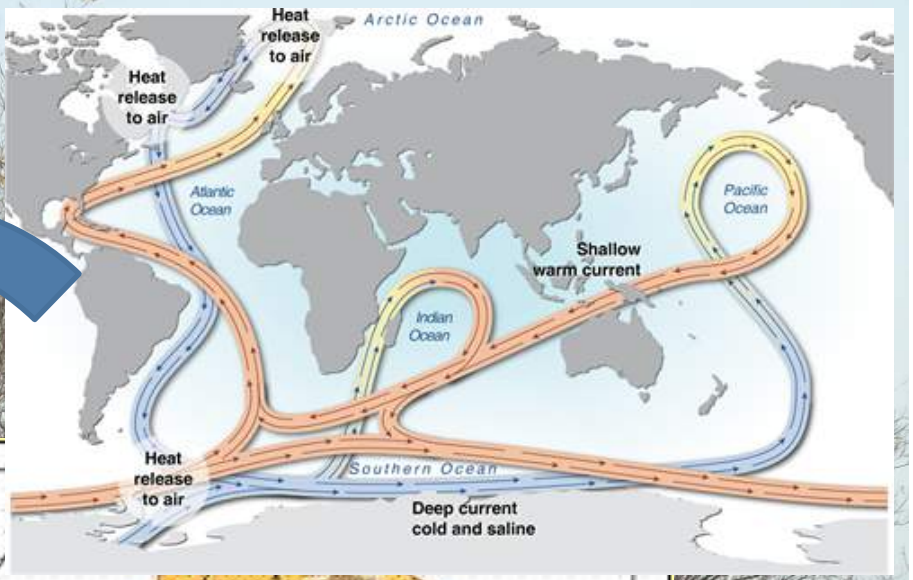
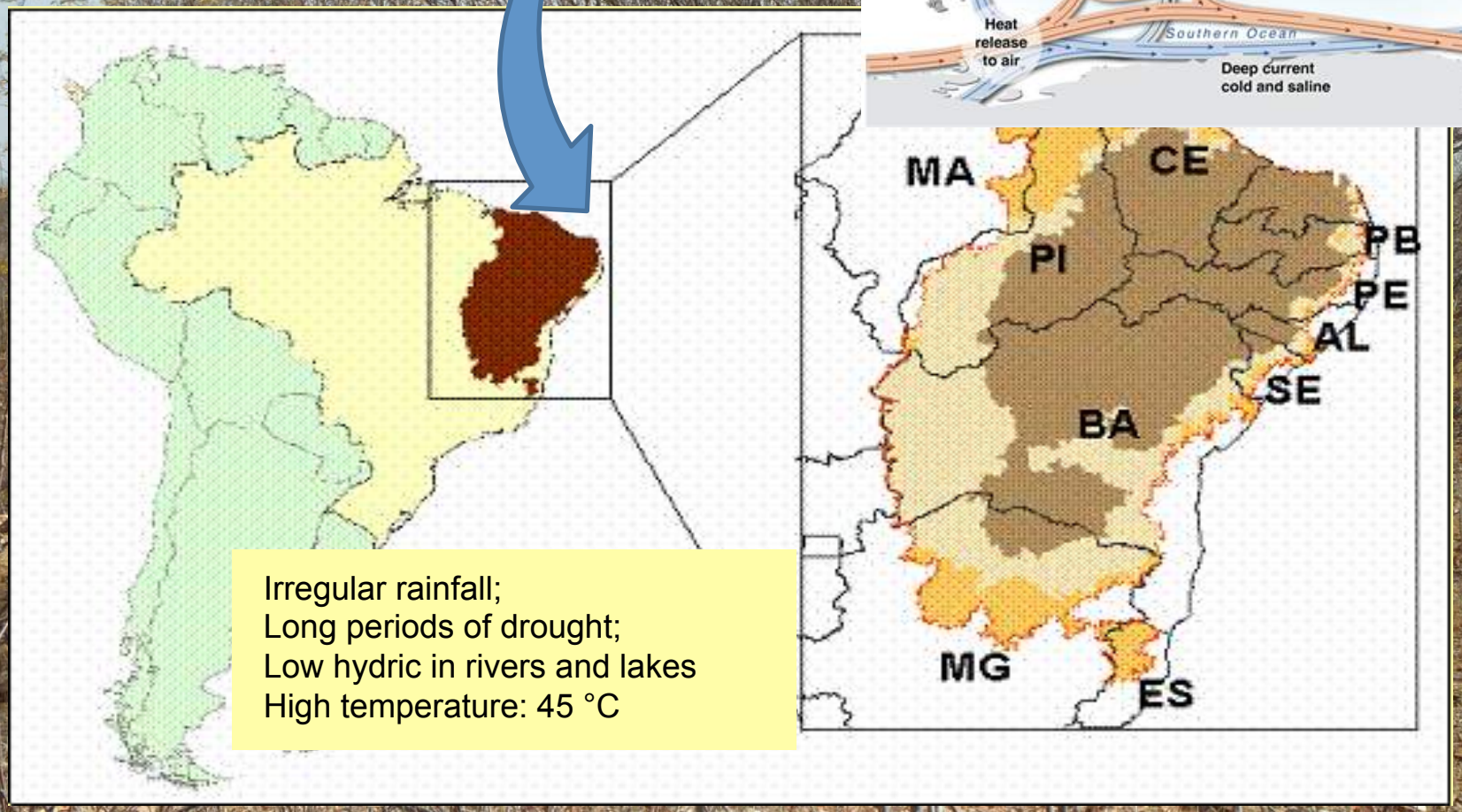
Extremófilos: Ecologia e Aplicações Biotecnológicas

Um olhar de bioprospecção para Caatinga: Singular Bioma Brasileiro

Suikinai Nobre Santos
suikinai@gmail.com

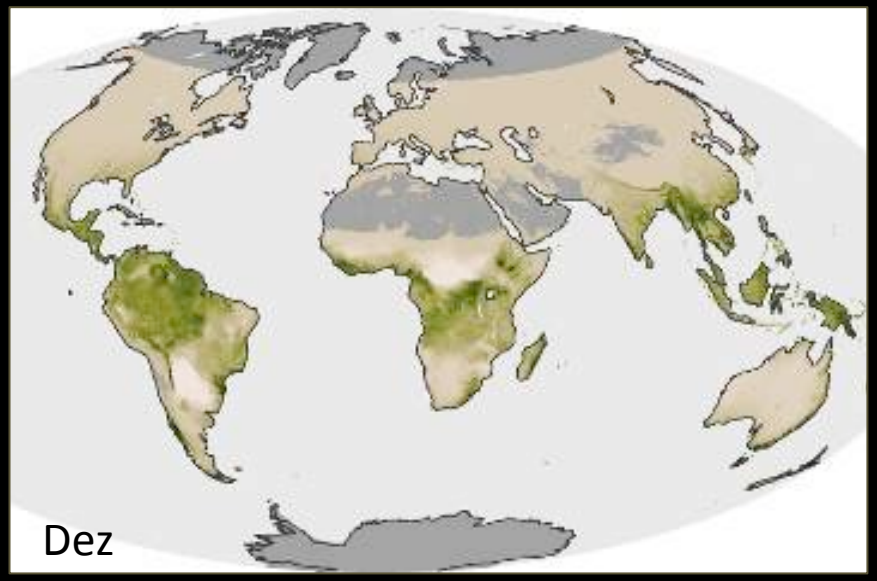
Caatinga

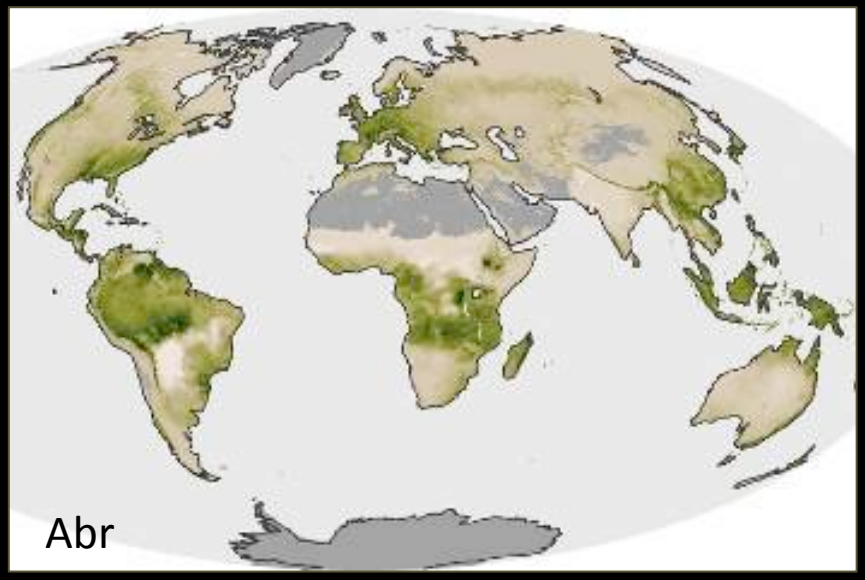


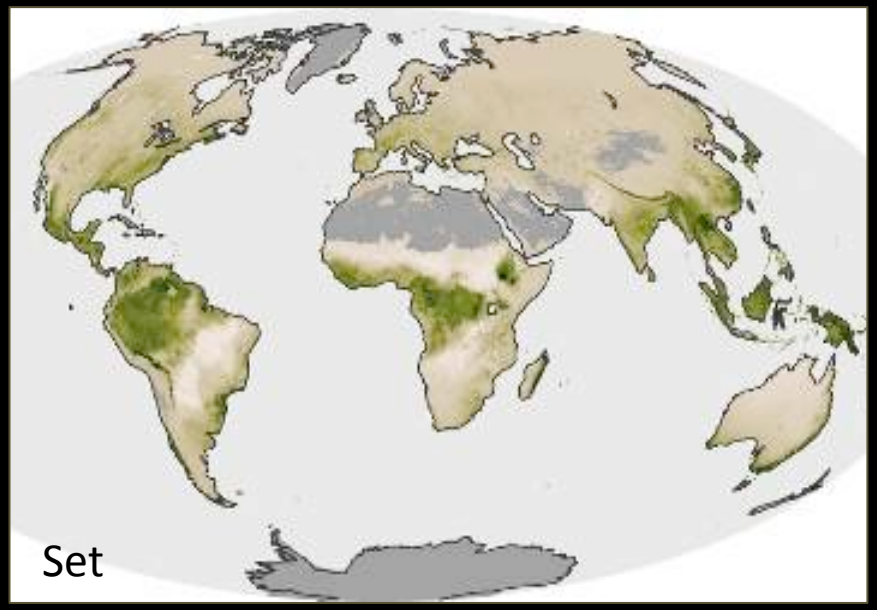


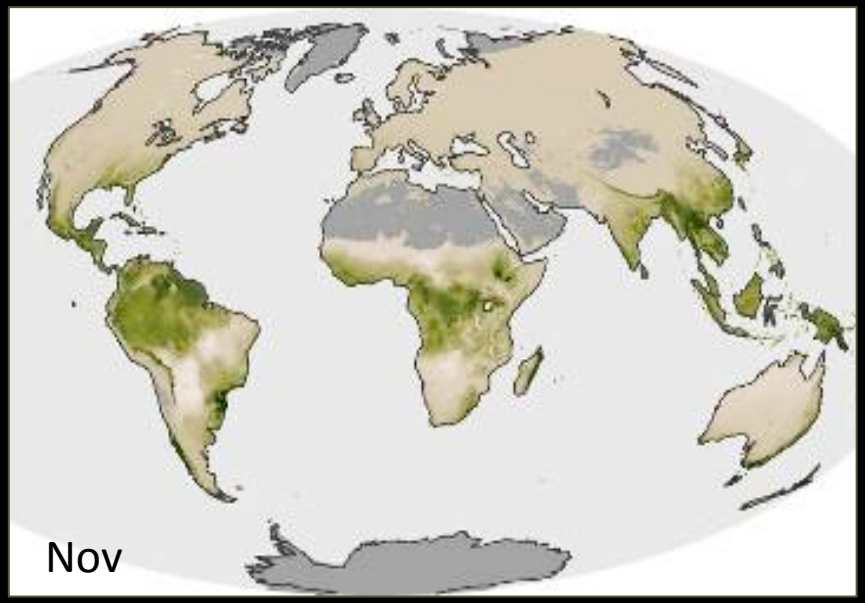
Irregular rainfall;
Long periods of drought;
Low hydric in rivers and lakes
High temperature: 45 °C

Metabolism” of Earth’s vegetation

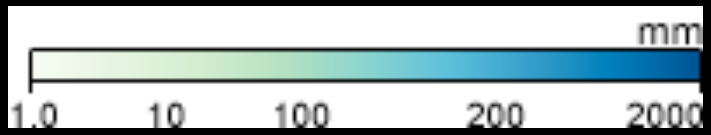


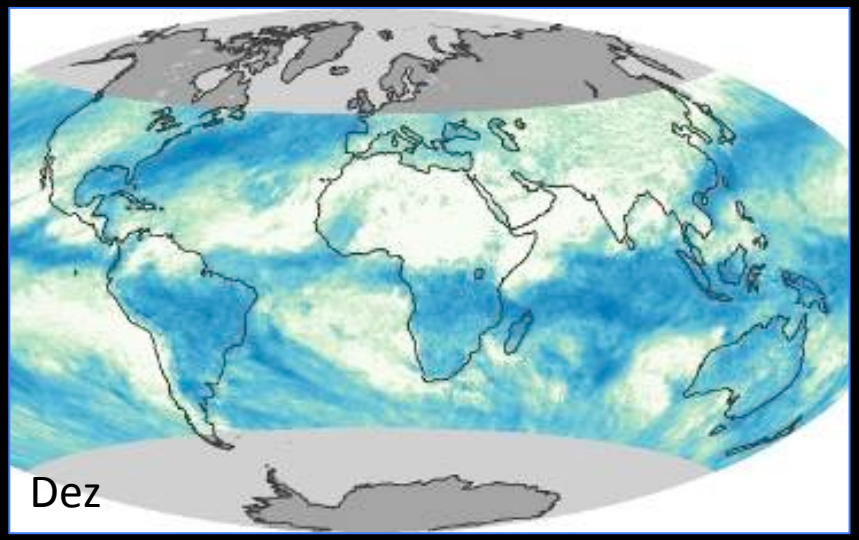


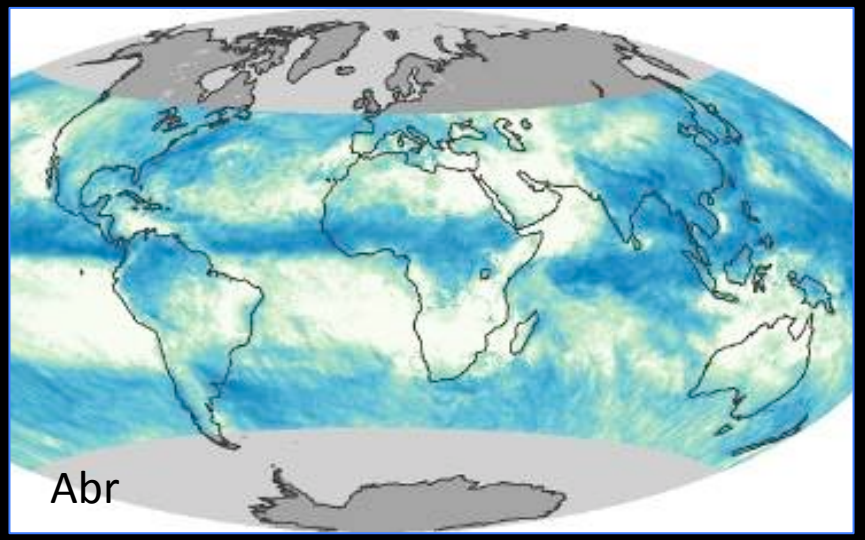


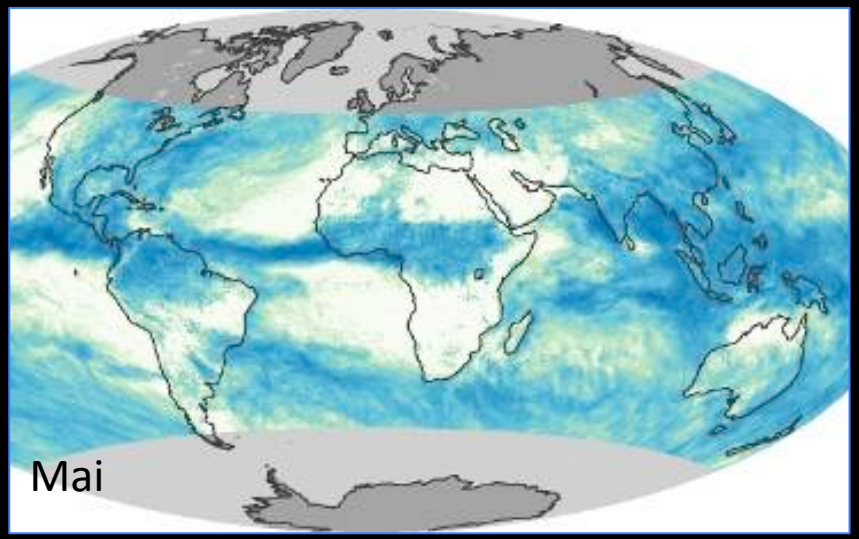


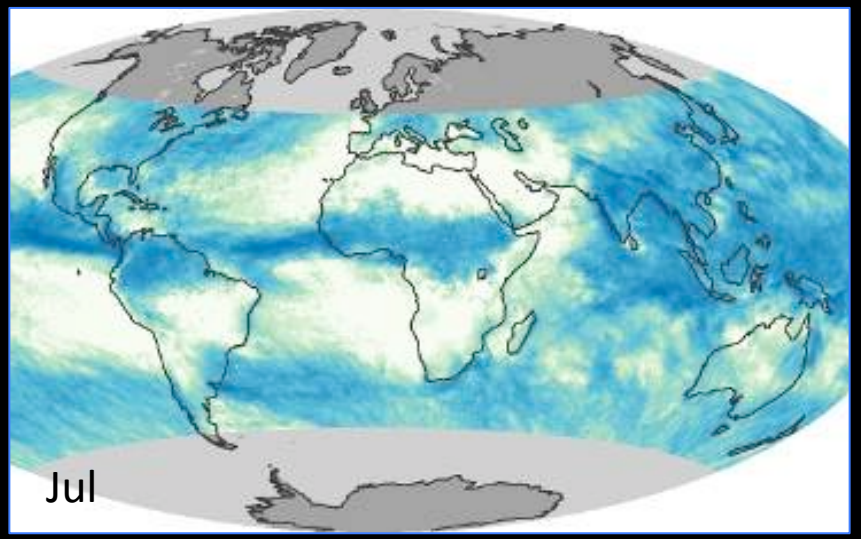
Total Rainfall



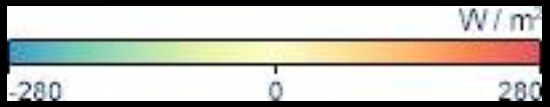


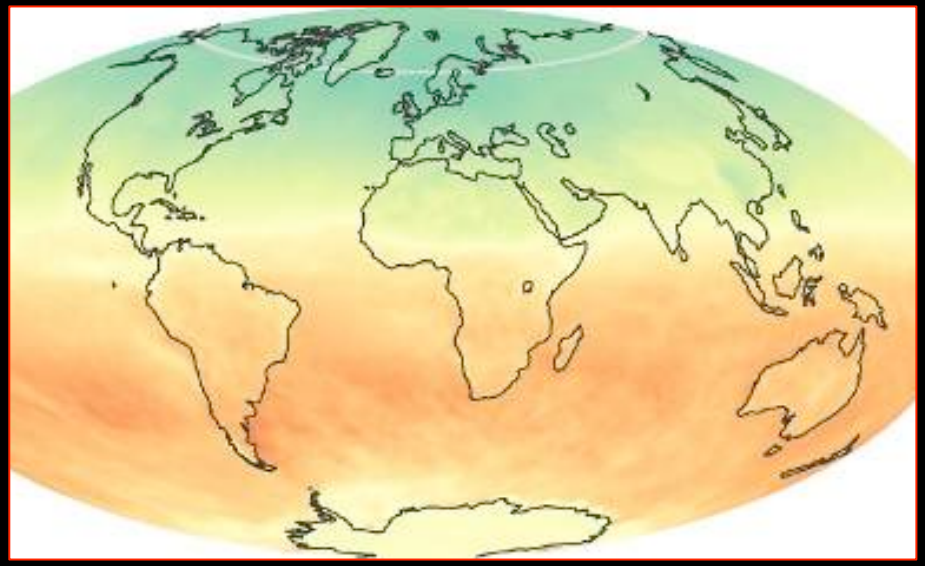


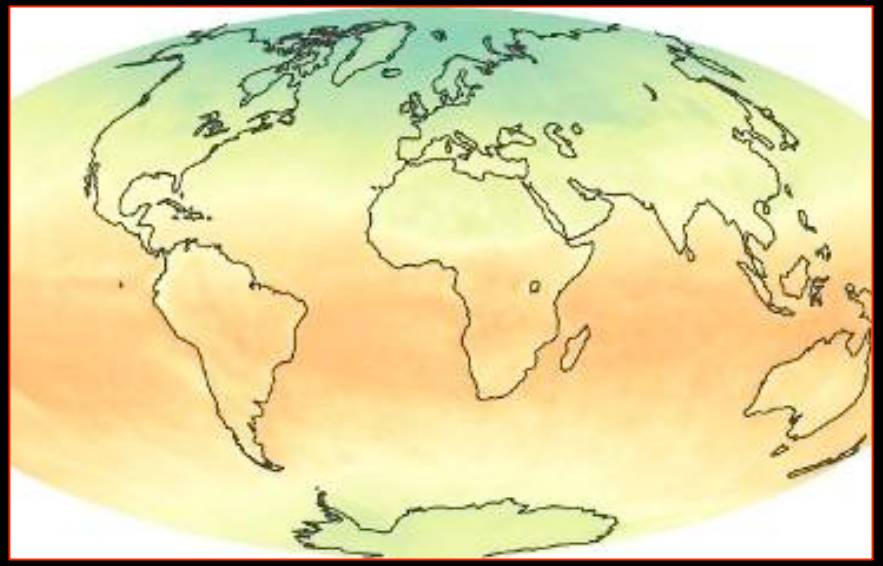


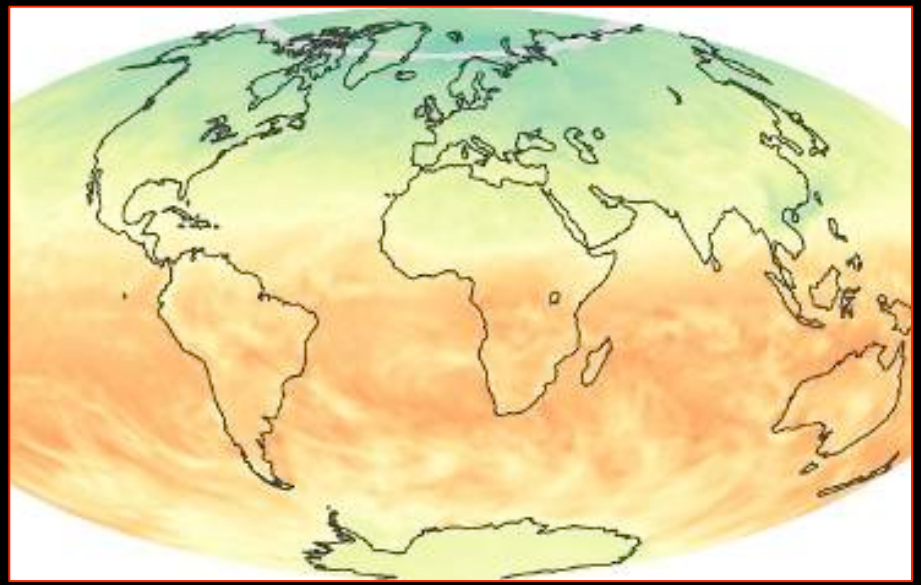


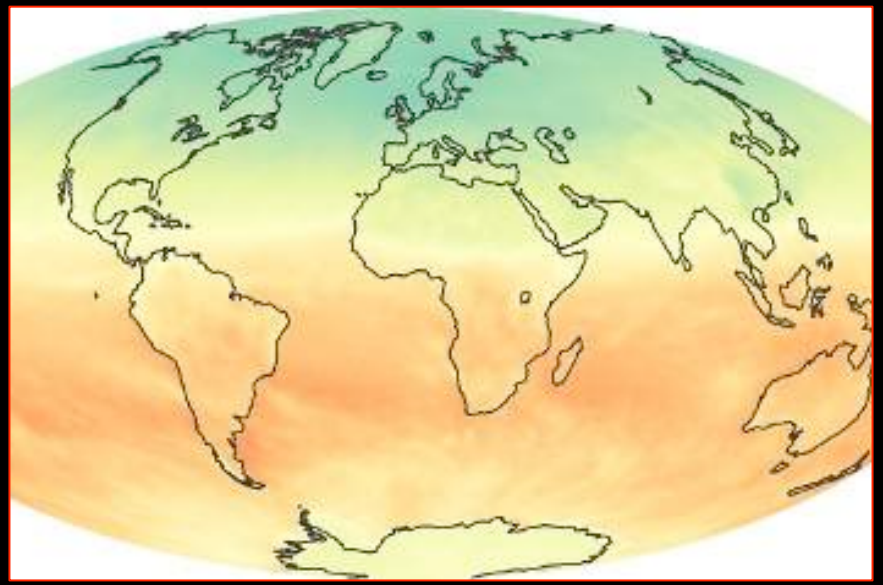
Net Radiation



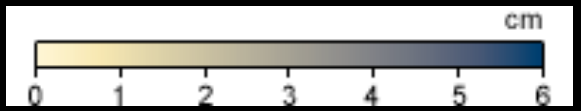


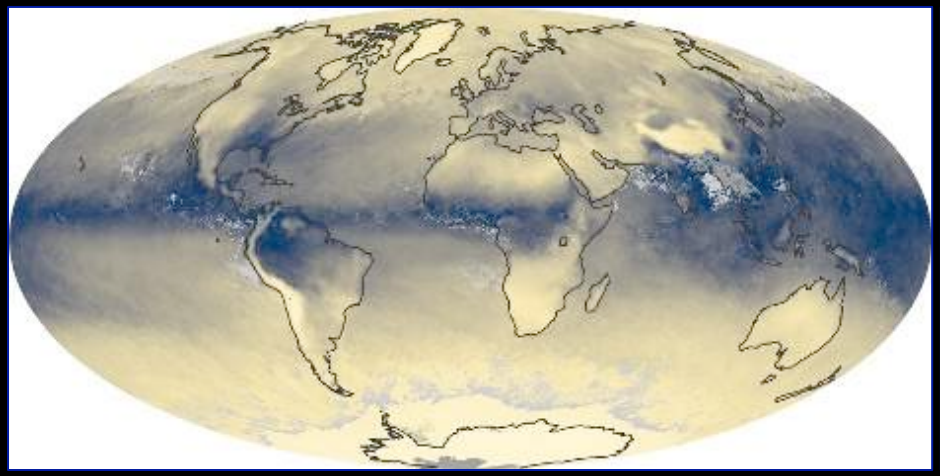


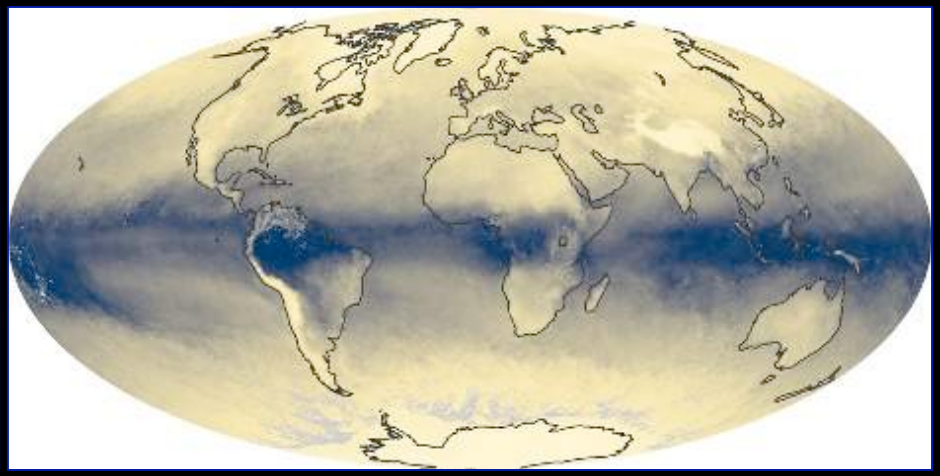


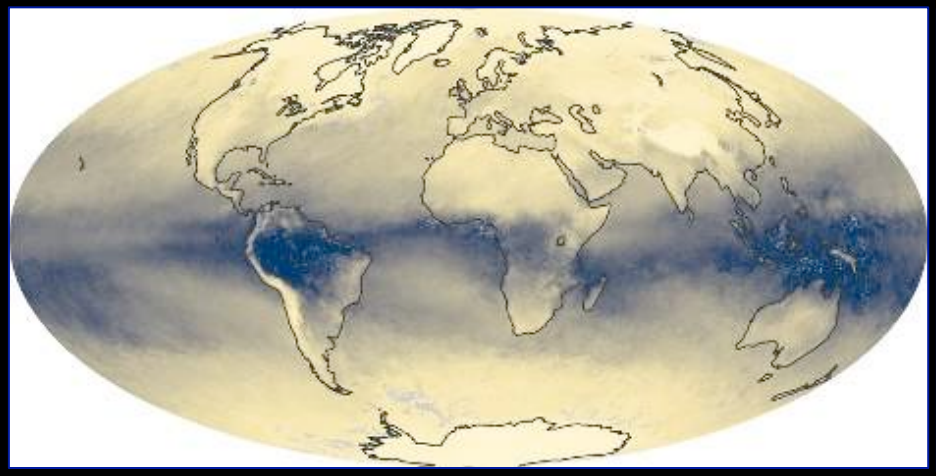


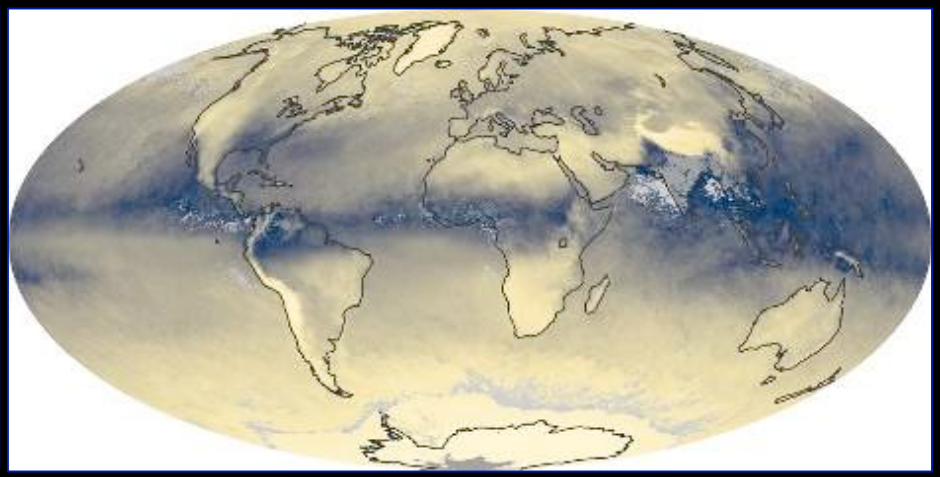
Water Vapor



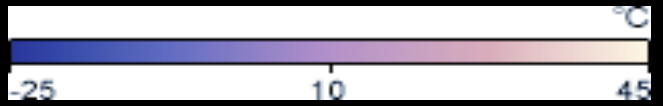


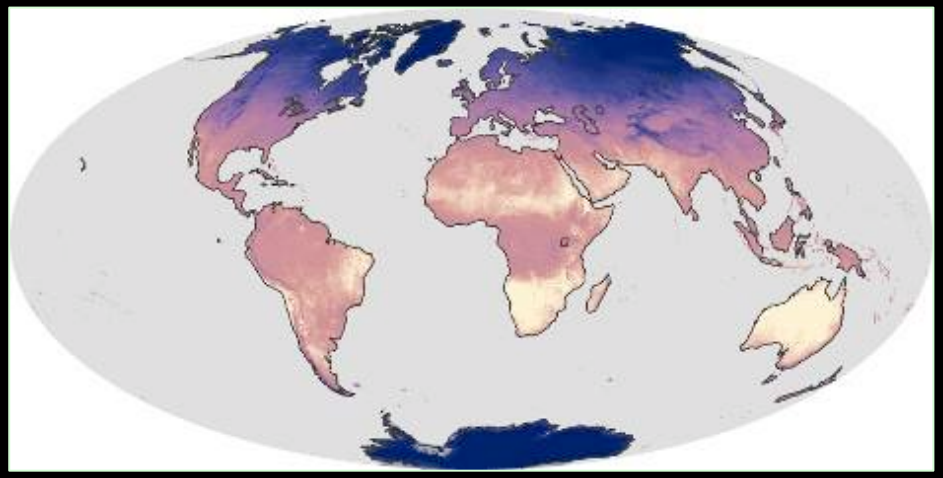


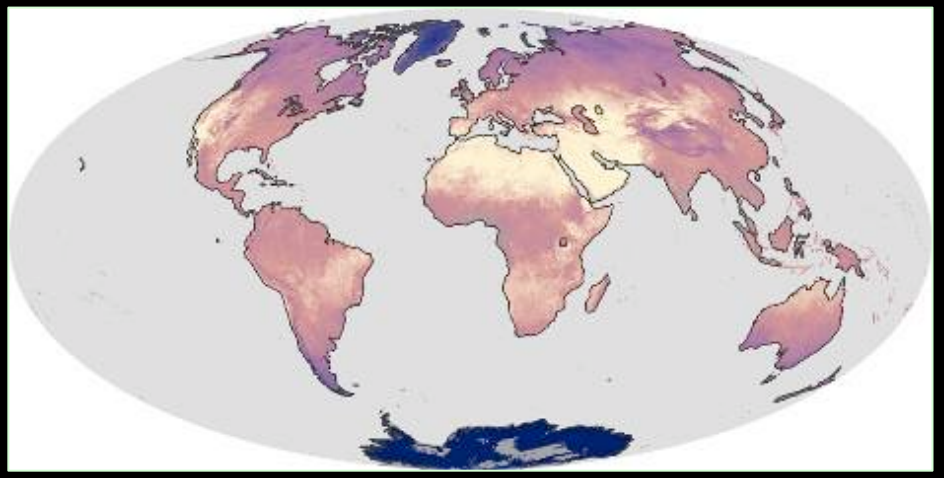


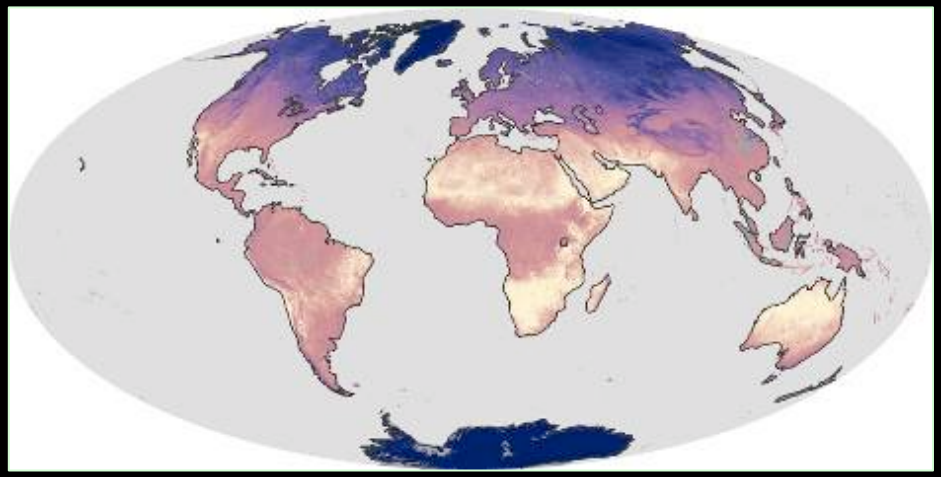


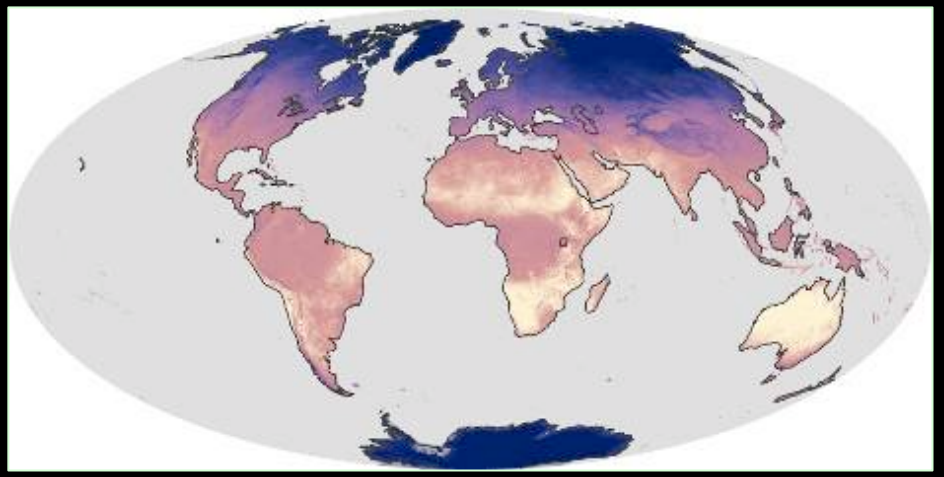
Land Surface Temperature





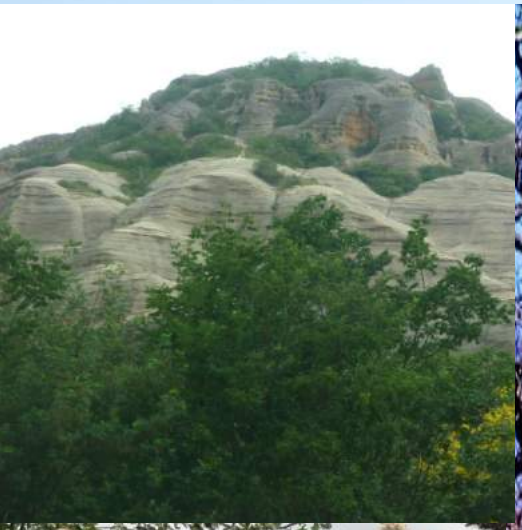






Caatinga of the semiarid regions: Rainy and dry season

Caatinga “White forest”: Extremophile area in Brazil



National Aeronautics and Space Administration

Global Nutrient Limitation In Terrestrial Vegetation

NASA has determined the limits to vegetation growth from soil nutrients availability

Using 19 years of remote sensing data, a research team led by Dr. Josh Fisher (JPL) calculated maximum possible vegetation productivity – as determined by available water and light – then cross-compared that theoretical maximum with observed vegetation productivity. Where actual vegetation productivity was less than the theoretical maximum, the researchers found vegetation was nutrient limited, after accounting for disturbance.

Globally, nutrient limitation reduces plant growth by 16-28%, on average.

The nutrient limitation maps significantly advance global carbon cycle and climate models, which struggle to characterize the role of nutrient cycling in limiting the ability of the terrestrial biosphere to take up CO_2.

The global study detected large scale nutrient gradients – an East - West gradient across Amazonia, fertilization differences between 'developed' and 'developing' countries, tree line migration boundaries in boreal North America, and distinctions between biomes such as forests, savannas, and grasslands.

Plant growth is enhanced or limited by the amounts of nutrients in the soil.

www.nasa.gov

Joshua B. Fisher | Jet Propulsion Laboratory & California Institute of Technology
Grayson Badgley | Stanford University
Eleanor Blyth | UK Centre for Ecology & Hydrology

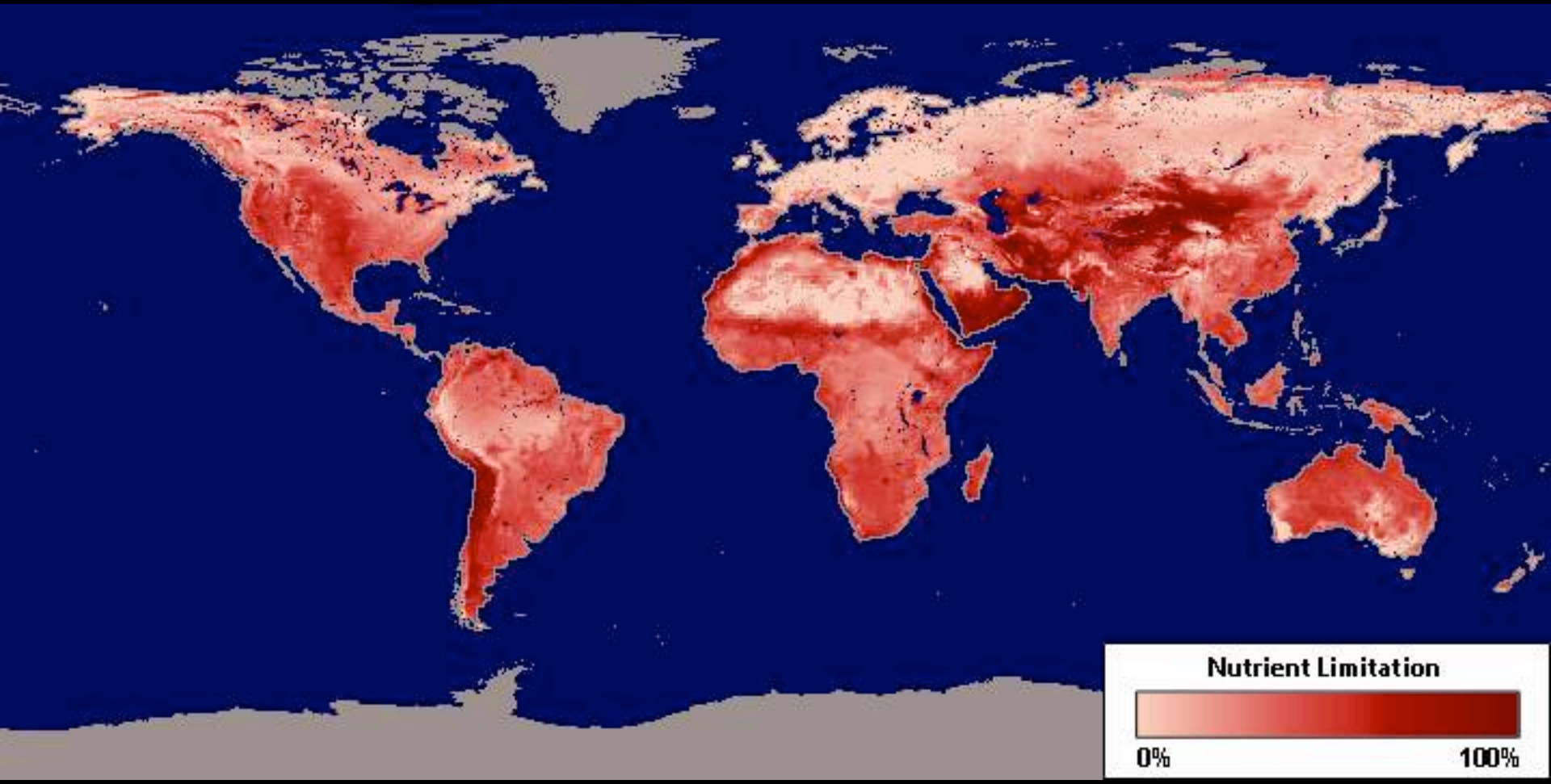
Floresta de caatinga

- 3 a 4 meses de chuvas;
- < Oferta de materia orgânica;
- Ciclagem limitada na época chuvosa

< Disponibilidade de nutriente

< Disponibilidade de água

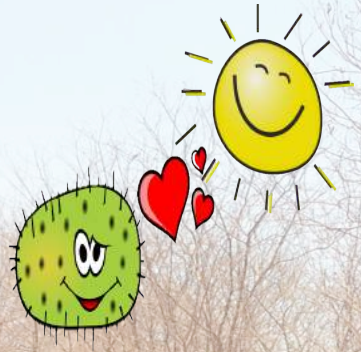
Global map depicting the percentage that vegetation growth is limited by available soil nutrients, with 0 representing no nutrient limitation, and 100 being completely nutrient limited. Image credit: NASA/JPL-Caltech, Jun, 2017.



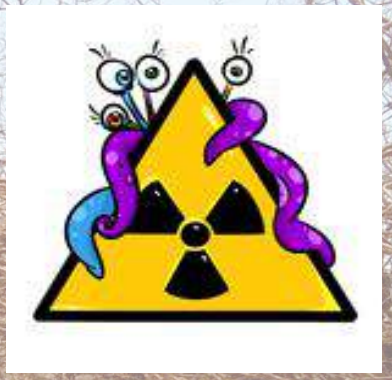
Estratégias:

HALOFÍLICOS....

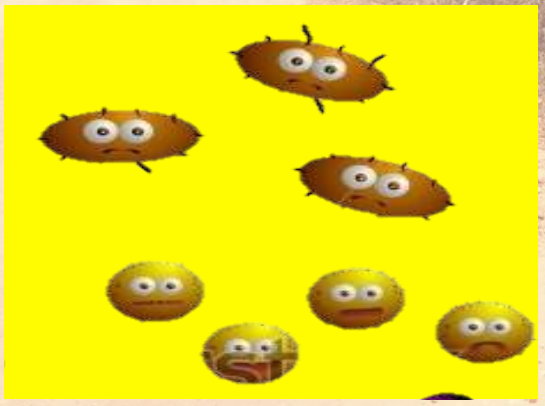
TERMOFÍLICOS



RADIORESISTENTES



XEROFÍLICOS



OSMOFÍLICOS

Aplicações Biotecnológicas

- Celulases: **Endoglucanases e β-glucosidades**
- Esterases e lipases
- Pectinases
- Proteases e Lipases (Detergentes e produtos diários)
- Fosfatase
- Aminopeptidases
- Lacases
- Peroxidases
- Surfactantes
- **Polimeros**
- Lipídios
- **B- carotenos** (industria de alimentos)
- Nucleases E amilases
- Álcool desidrogenase (síntese química)
- Xilanases (papel bleaching)
- Bacteriorodopsina (Colírio)
- **Compatíveis solúveis** (proteção contra
- congelamento aquecimento)
- **Antibióticos**
- **Pigmentos**
- Linhagens Promotoras de crescimentos
- Substitutos de agrotóxicos

Biological Source

GENETIC

Transformed into mutants OGM

AGRICULTURE

Biological Control

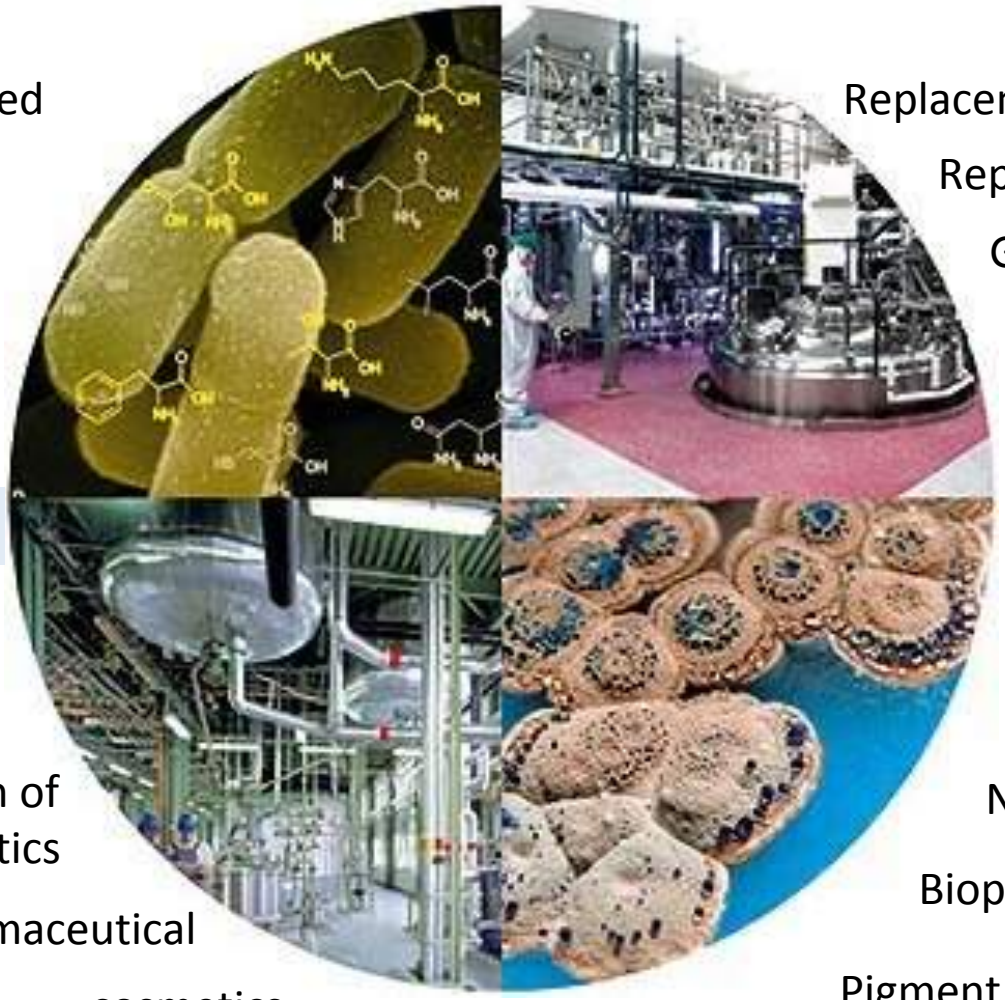
Replacement of Pesticides

Replacement of Herbicides

Growth promoters

Inoculants

Recovery areas desertification



INDUSTRIAL

Production of biofuels

Production of green plastics

Pharmaceutical

cosmetics

BIOTECHNOLOGY

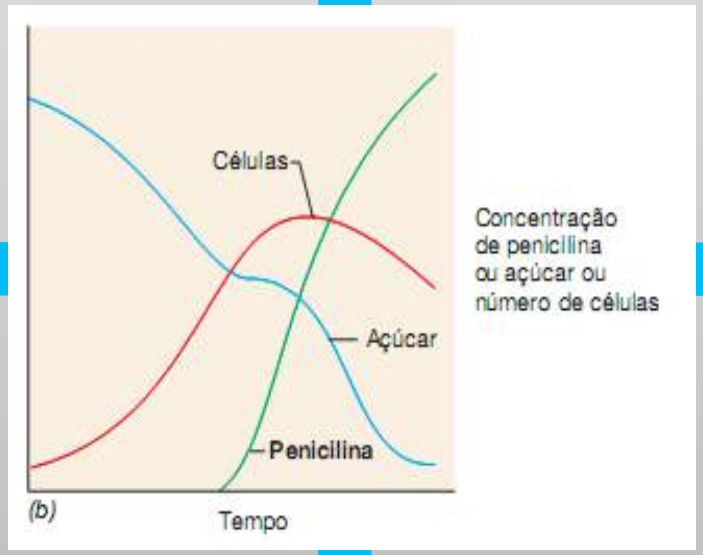
Antibiótic

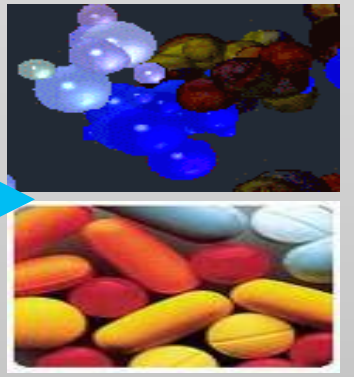
N Antibiótics

Biopolymers Production

Pigment Production

Metabolims Primary





scientific importance

ecological importance

industrial importance

Secondary Metabolism

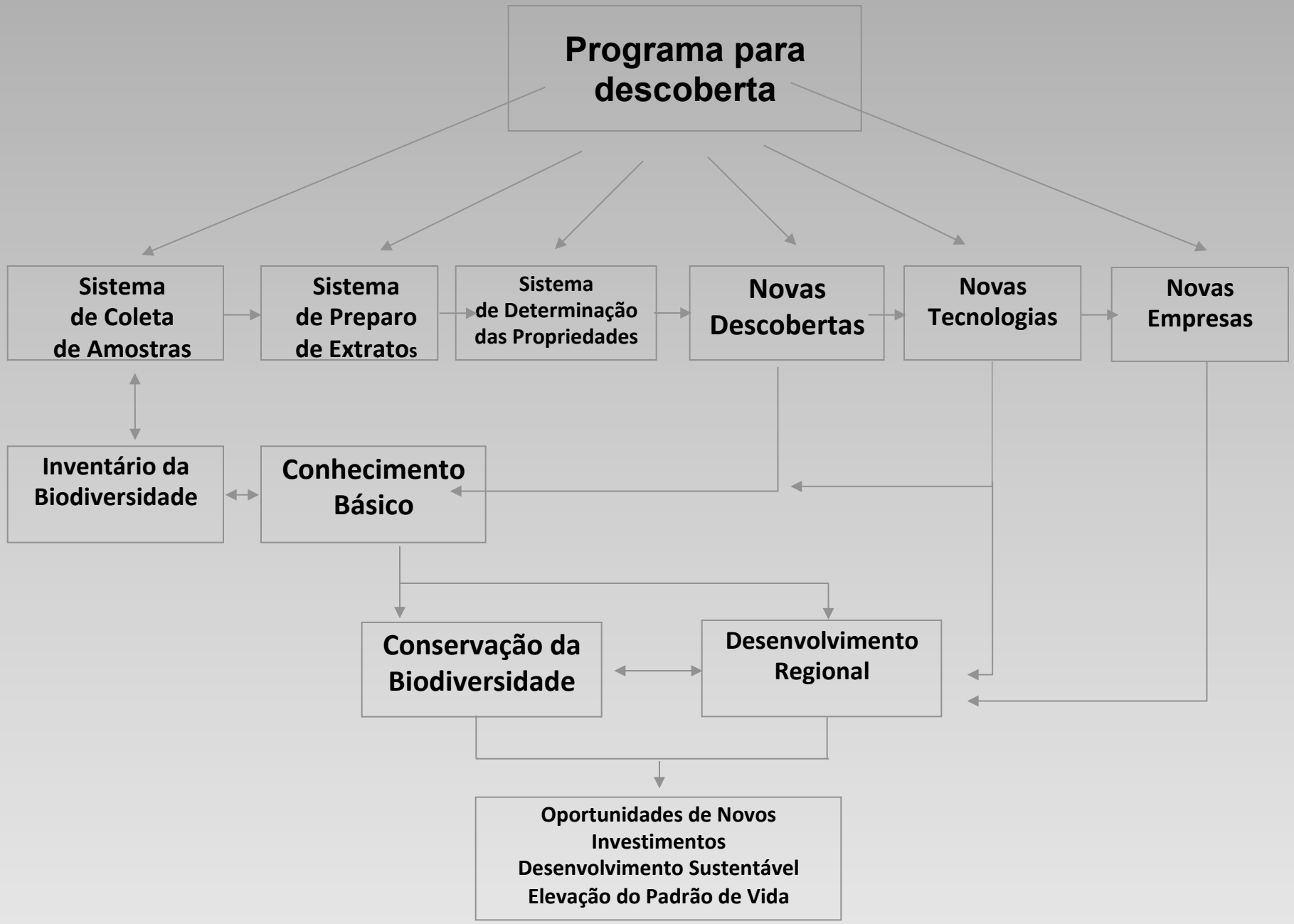
chemically diverse substances

Natural Product

Bioprospecting

Biodiversity

- Reservoir for the discovery of new molecules



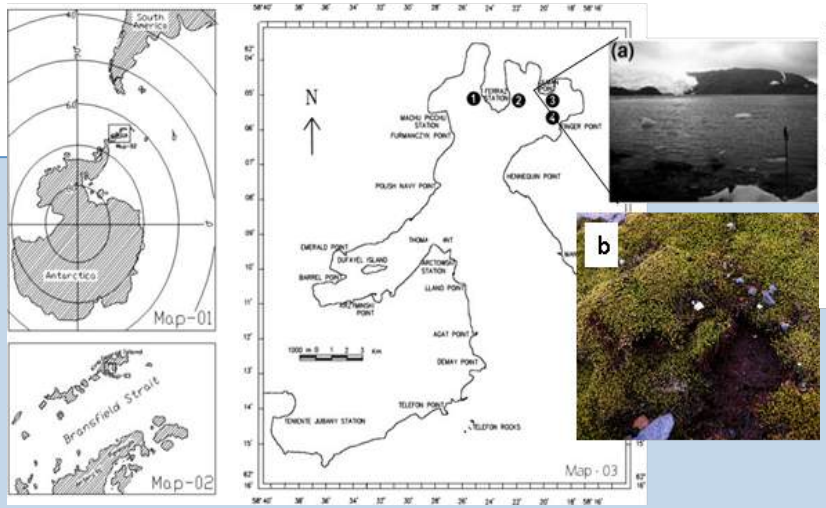
Representação esquemática dos procedimentos globais para bioprospecção de produtos naturais

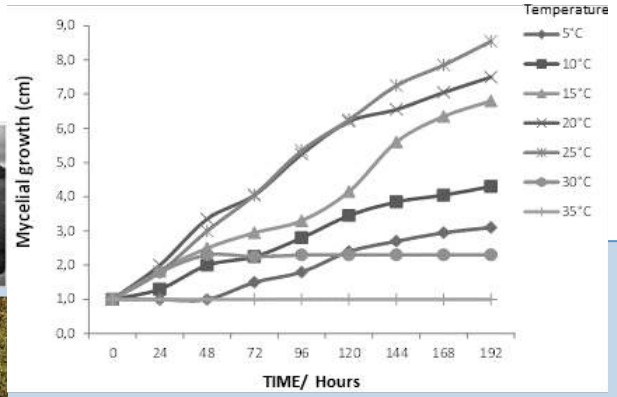
Extremophiles
DOI 10.1007/s00792-013-0588-7

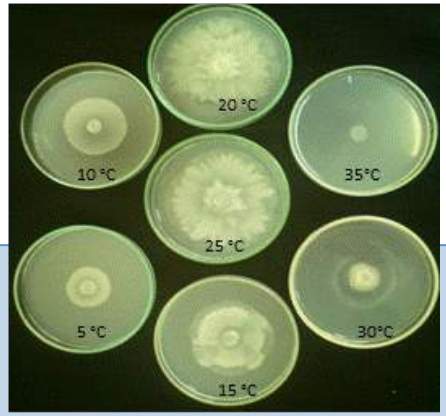
ORIGINAL PAPER

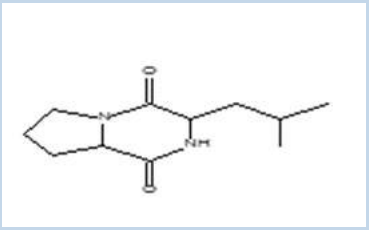
Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss *Schistidium antarctici*

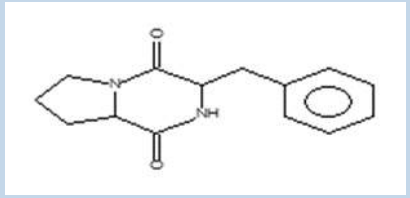
Itamar S. Melo · Suikinai N. Santos · Luiz H. Rosa · Marcia M. Parma · Leonardo J. Silva · Sonia C. N. Queiroz · Vivian H. Pellizari











Pyrrolo [1,2-a] pyrazine -1,4-doine, hexahydro - 3- (phenylmethyl).

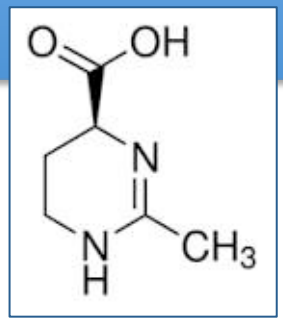
HALOTOLERANT BACTERIA: isolated halotolerantes plants in the Brazilian semiarid region: Promising production of ECTOINE

Blutaparon portulacoides

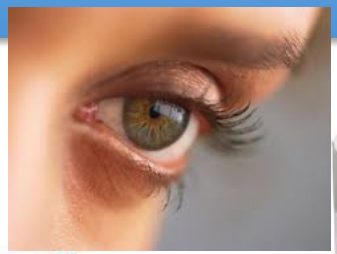
***Spergularia* sp.**

How Halophiles survive high salt concentrations??

- Many small proteins or particles inside of the cells..
Osmotics balance



Ectoine

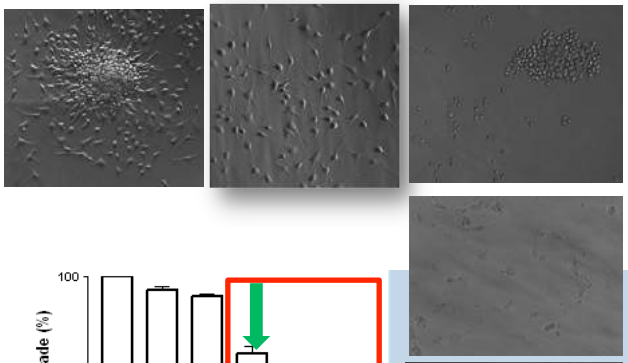


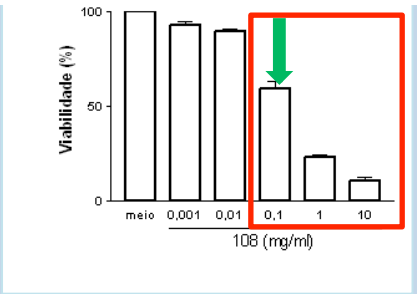
EYE DROP

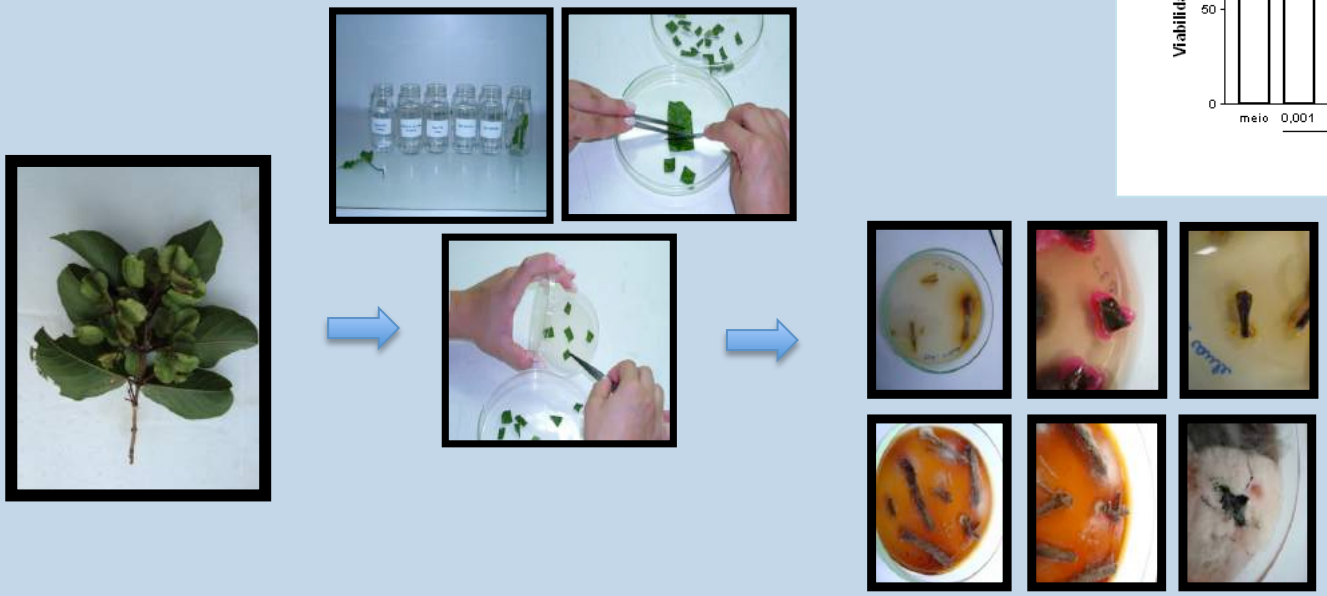
Symbiosis
DOI 10.1007/s13199-012-0218-7

Endophytic fungi from *Combretum leprosum* with potential anticancer and antifungal activity

Suikinai Nobre Santos · Faustos Klabund Ferraris · Ana Olivia de Souza · Maria das Graças Henriques · Itamar Soares Melo







Metagenomica Funcional: Estratégia para acessar metagenoma do solo por meio da construção e screening de bibliotecas de DNA

Traduzido e adaptado de: RESEARCH COUNCIL (U.S.). COMMITTEE ON METAGENOMICS: CHALLENGES AND FUNCTIONAL APPLICATIONS, 2007

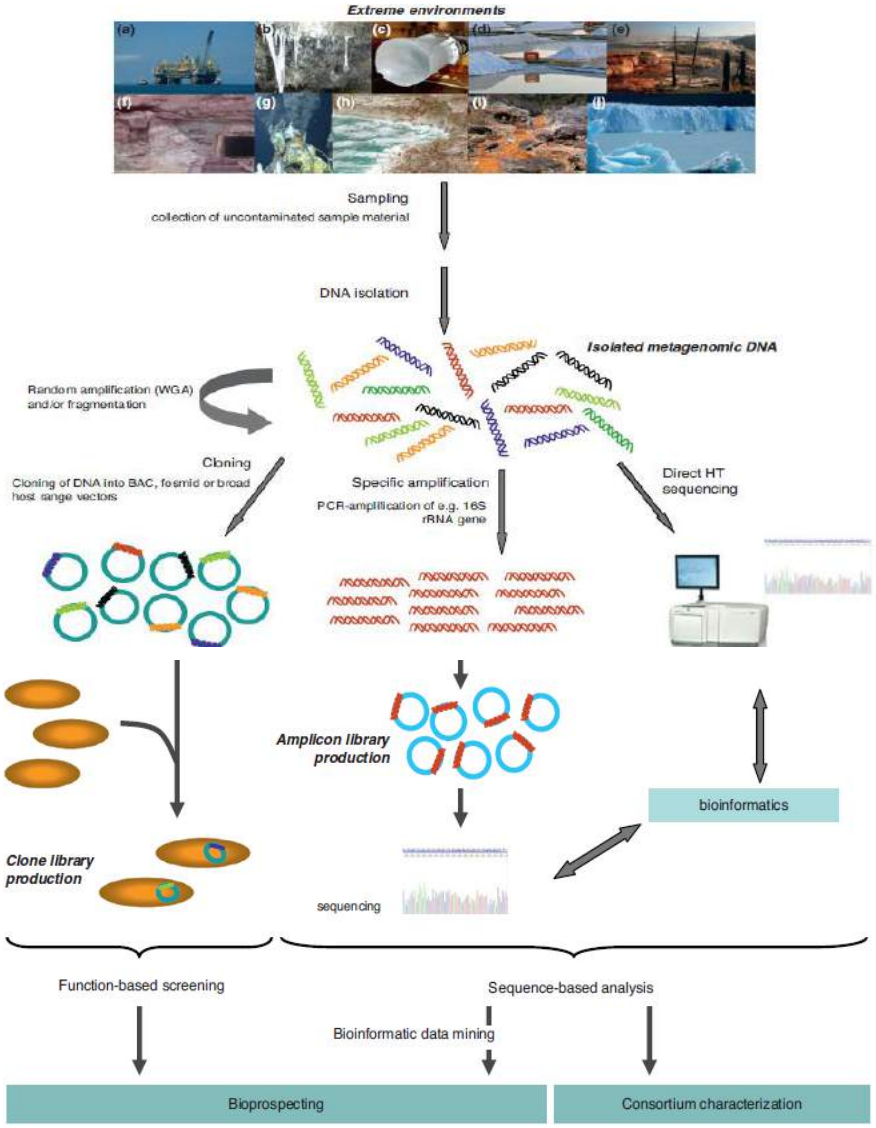
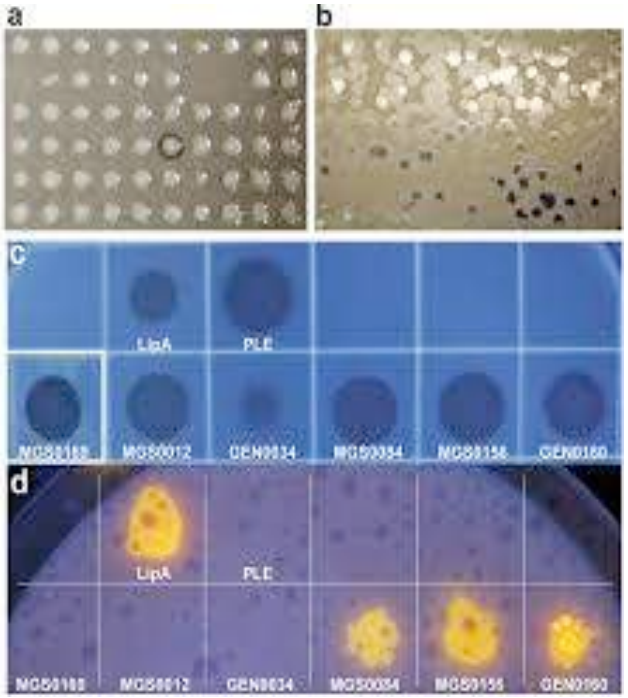


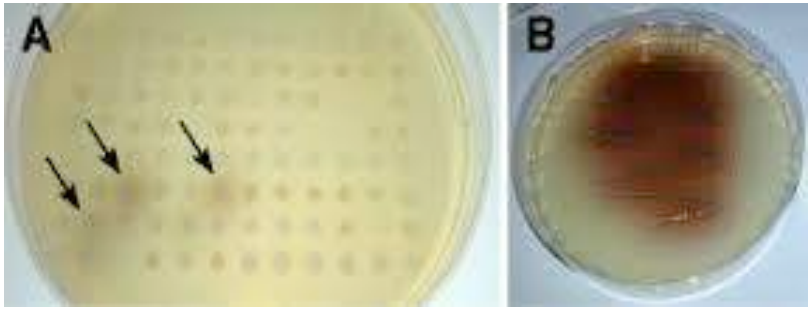
Figura 1. Esquema da metodologia geral de estudo metagenômico. Fonte: Lewin et al. (2013).

Metagenoma de solos da caatinga_MG_Rast

location	start	stop	strand	function	aliases	figfam	evidence_codes
_contig_1_35_1231	35	1231	+	hypothetical protein			
_contig_1_1762_1541	1762	1541	-	hypothetical protein			
_contig_1_3174_1990	3174	1990	-	hypothetical protein			
_contig_1_3364_3966	3364	3966	+	Cation transport protein chaC			
_contig_1_4168_4410	4168	4410	+	hypothetical protein			
_contig_1_4471_5895	4471	5895	+	Nodulation protein noeA			
_contig_1_5892_7466	5892	7466	+	Choline-sulfatase (EC 3.1.6.6)			isu;Choline_and_Betaine_Uptake_and_Betaine_Biosynthesis
_contig_1_7661_8773	7661	8773	+	hypothetical protein			
_contig_1_9599_8811	9599	8811	-	1-acyl-sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51)			
_contig_1_10300_9596	10300	9596	-	YdcF-like protein			
_contig_1_11342_10374	11342	10374	-	Cell division protein FtsX			
_contig_1_11970_11335	11970	11335	-	Cell division transporter, ATP-binding protein FtsE (TC 3.A.5.1.1)			
_contig_1_12298_13068	12298	13068	+	bll1389; hypothetical protein			
_contig_1_13102_13476	13102	13476	+	Two-component response regulator			
_contig_1_13607_13843	13607	13843	+	hypothetical protein			
_contig_1_16557_13945	16557	13945	-	Hypothetical protein			
_contig_1_16487_17245	16487	17245	+	Small Subunit Ribosomal RNA; ssuRNA; SSU rRNA			
_contig_1_16740_17123	16740	17123	+	4-carboxymuconolactone decarboxylase (EC 4.1.1.44)			isu;Pyrene_degradation isu;Protocatechuate_branch_of_beta-ketoad
_contig_1_18362_18826	18362	18826	+	hypothetical protein			
_contig_2_457_1803	457	1803	+	Type I secretion outer membrane protein, TolC precursor			isu;Multidrug_Resistance_Efflux_Pumps
_contig_2_1897_2502	1897	2502	+	Mll1106 protein			
_contig_2_3023_2499	3023	2499	-	hypothetical protein			
_contig_2_3197_4408	3197	4408	+	Valyl-tRNA synthetase (EC 6.1.1.9)			idu(1);tRNA_aminoacylation,_Val
_contig_3_195_49	195	49	-	hypothetical protein			
_contig_3_232_1512	232	1512	+	Valyl-tRNA synthetase (EC 6.1.1.9)			idu(1);tRNA_aminoacylation,_Val
_contig_3_1663_4293	1663	4293	+	bll5864; hypothetical protein			
_contig_3_4290_4556	4290	4556	+	Sensory box/GGDEF family protein			
_contig_3_4817_4575	4817	4575	-	hypothetical protein			
_contig_3_6414_5005	6414	5005	-	Glutamine synthetase type I (EC 6.3.1.2)			isu;Glutamine,_Glutamate,_Aspartate_and_Asparagine_Biosynthesis
_contig_3_6869_6531	6869	6531	-	Nitrogen regulatory protein P-II			icw(1);Ammonia_assimilation
_contig_3_7101_8678	7101	8678	+	NAD(P)HX epimerase / NAD(P)HX dehydratase			isu;YjeE isu;YjeE
_contig_3_8783_8971	8783	8971	+	hypothetical protein			
_contig_3_9073_9157	9073	9157	+	tRNA-Leu-TAG			
_contig_3_9202_10554	9202	10554	+	Cell division trigger factor (EC 5.2.1.8)			
_contig_3_10716_11180	10716	11180	+	ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)			icw(1);cAMP_signaling_in_bacteria icw(2);Proteolysis_in_bacteria,_A
_contig_3_11177_11368	11177	11368	+	ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)			icw(1);cAMP_signaling_in_bacteria icw(2);Proteolysis_in_bacteria,_A
_contig_3_11563_12828	11563	12828	+	ATP-dependent Clp protease ATP-binding subunit ClpX			isu;Proteolysis_in_bacteria,_ATP-dependent
_contig_3_12995_14851	12995	14851	+	ATP-dependent protease La (EC 3.4.21.53) Type I			icw(3);Proteolysis_in_bacteria,_ATP-dependent
_contig_3_14820_15101	14820	15101	+	Biosynthetic Aromatic amino acid aminotransferase beta (EC 2.6.1.57) @ Histidinol-phosphate aminotransferase (EC 2.6.1.9)			
_contig_3_15098_16042	15098	16042	+	Cyclohexadienyl dehydrogenase (EC 1.3.1.12)(EC 1.3.1.43) # TyrAc, NADP-specific			
_contig_3_16479_16036	16479	16036	-	bll0174; hypothetical protein			

Screening





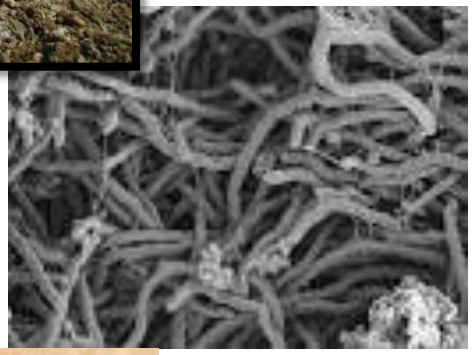
Lacases

L- Asparaginases

Bioprospecção em isolados

- Collect area
- Northeast of Brazil
- Dry season

Temperature around 40 – 50 °C



Crude extract from
Semi- solid medium
Fermentation after 7 days
grown, 28 celsios degreen

Fermentation: Farming, Filtration and Obtaining Raw extracts of strains selected

isolated preselected

Growth in 1000 mL
Czapek broth
stationary conditions
(28 °C/ 30 days

Extraction of metabolites

Concentration of the organic phase in rotaevaporator

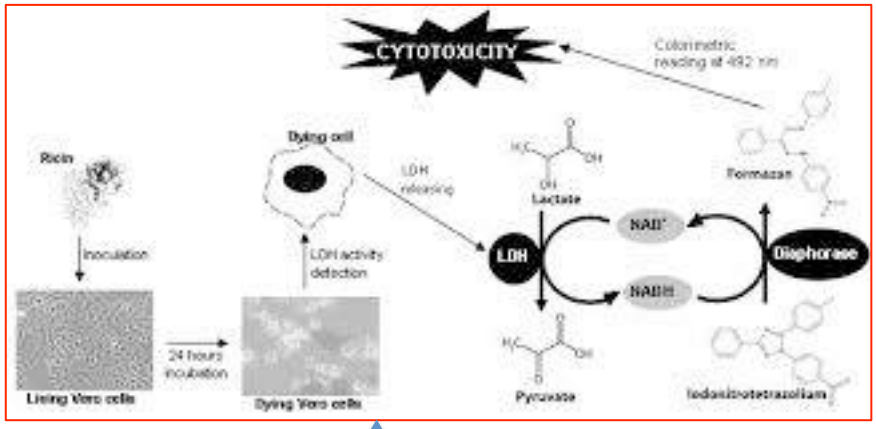
Crude extract

Successful metabolomic research requires effective metabolite extraction. For non-targeted metabolomics, extraction methods need to capture a broad range of cellular and biofluid metabolites, while excluding components such as proteins that are not intended for analysis. Extraction is made more challenging by the physico-chemical diversity of metabolites and by metabolite abundances that can vary by many orders of magnitude.

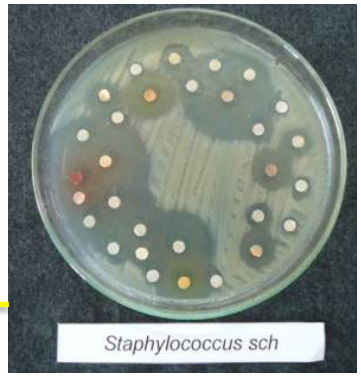
Authors

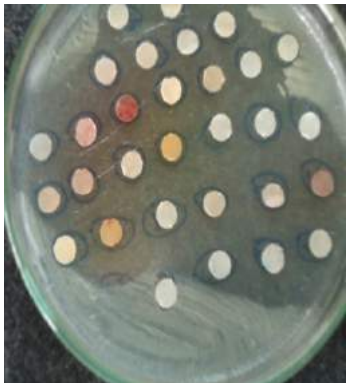
Theodore Sana and Steve Fischer

Agilent Technologies, Inc.,
Santa Clara, CA, USA

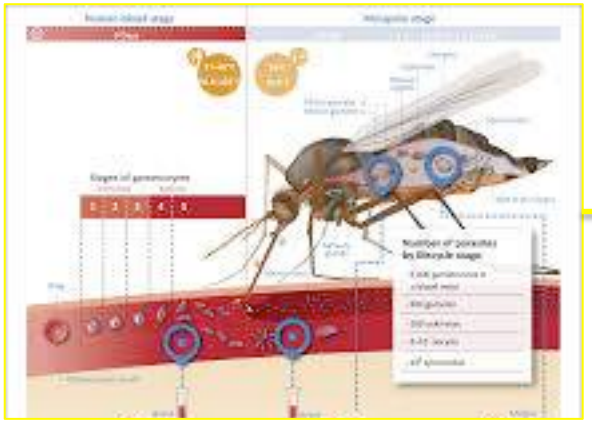


Antimicrobial Activity





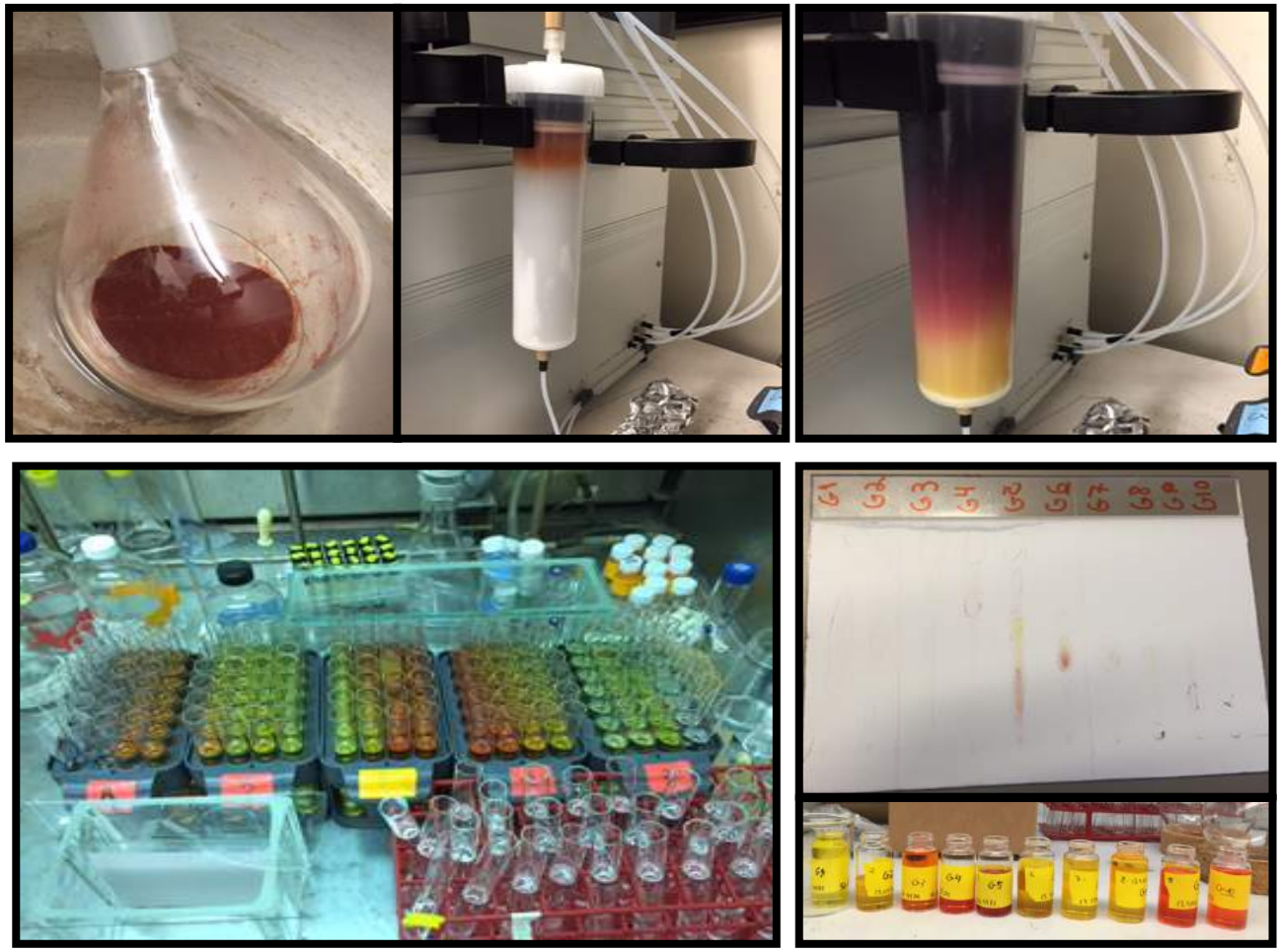
Screening crude extract

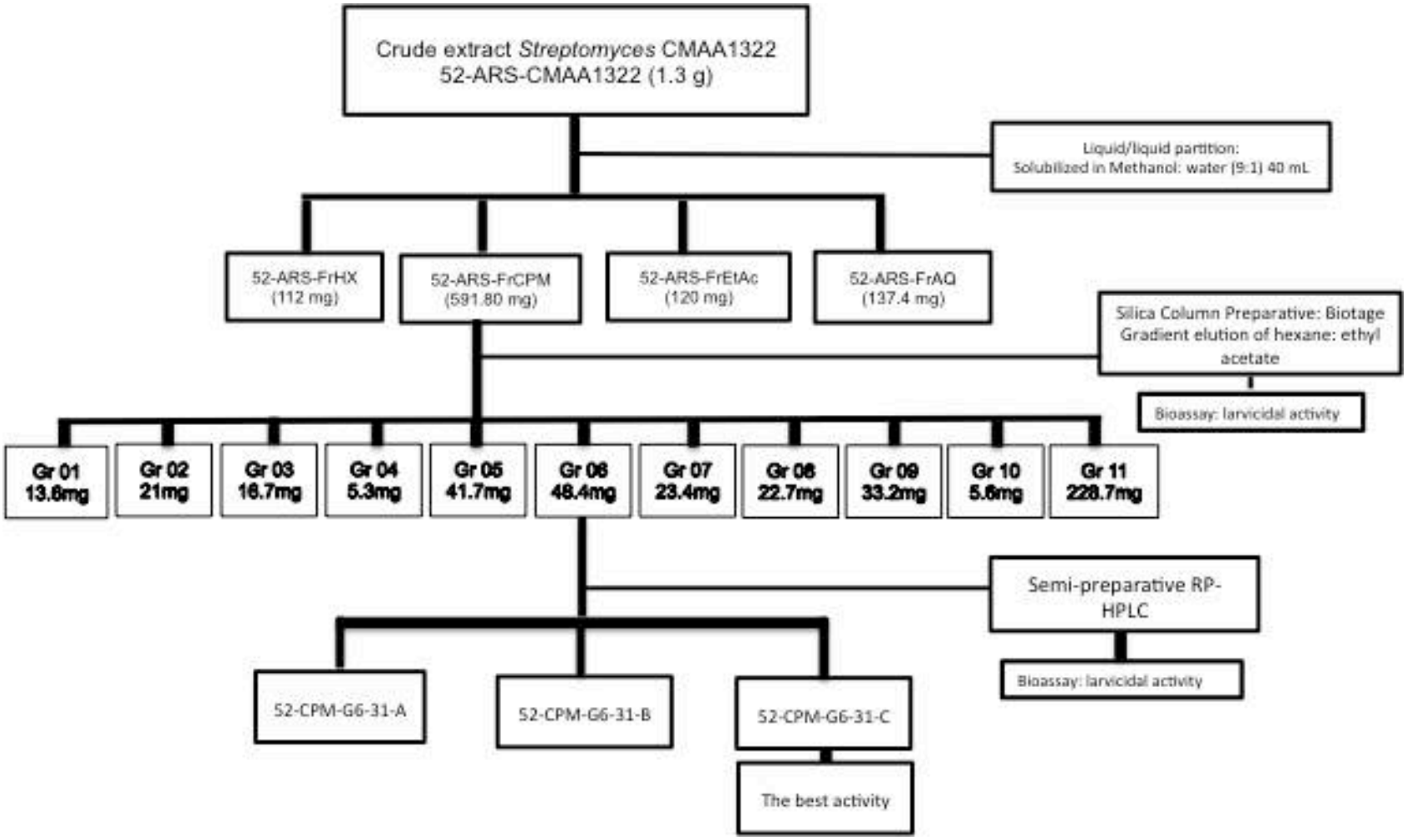


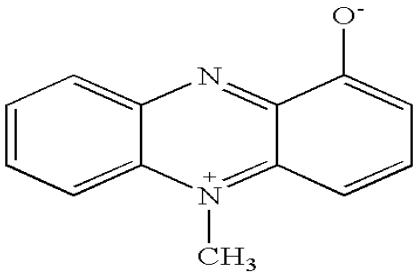
Mosquito Assay

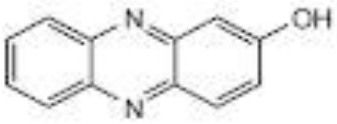
Processo de Purificação

Liquid Medium with
20 day of growth

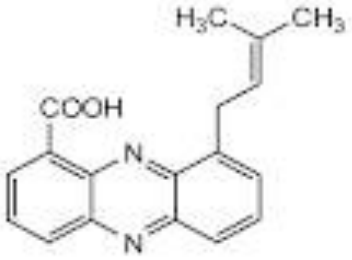



Crude extract *Streptomyces* CMAA1322
52-ARS-CMAA1322 (1.3 g)
Liquid/liquid partition:
Solubilized in Methanol: water (9:1) 40 mL
52-ARS-FrHX
(112 mg)
52-ARS-FrCPM
(591.80 mg)
52-ARS-FrEtAc
(120 mg)
52-ARS-FrAQ
(137.4 mg)
Silica Column Preparative: Biotage
Gradient elution of hexane: ethyl acetate
Bioassay: larvicidal activity
Gr 01
13.6mg
Gr 02
21mg
Gr 03
16.7mg
Gr 04
5.3mg
Gr 05
41.7mg
Gr 06
48.4mg
Gr 07
23.4mg
Gr 08
22.7mg
Gr 09
33.2mg
Gr 10
5.6mg
Gr 11
228.7mg
Semi-preparative RP-HPLC
Bioassay: larvicidal activity
52-CPM-G6-31-A
52-CPM-G6-31-B
52-CPM-G6-31-C
The best activity

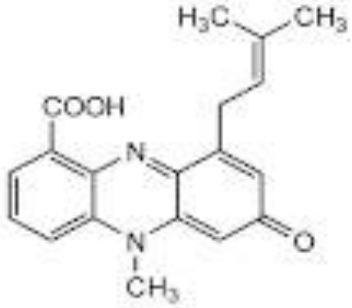




2-Hydroxy-phenazine



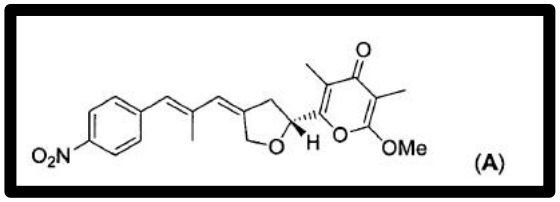
Endophenazine A



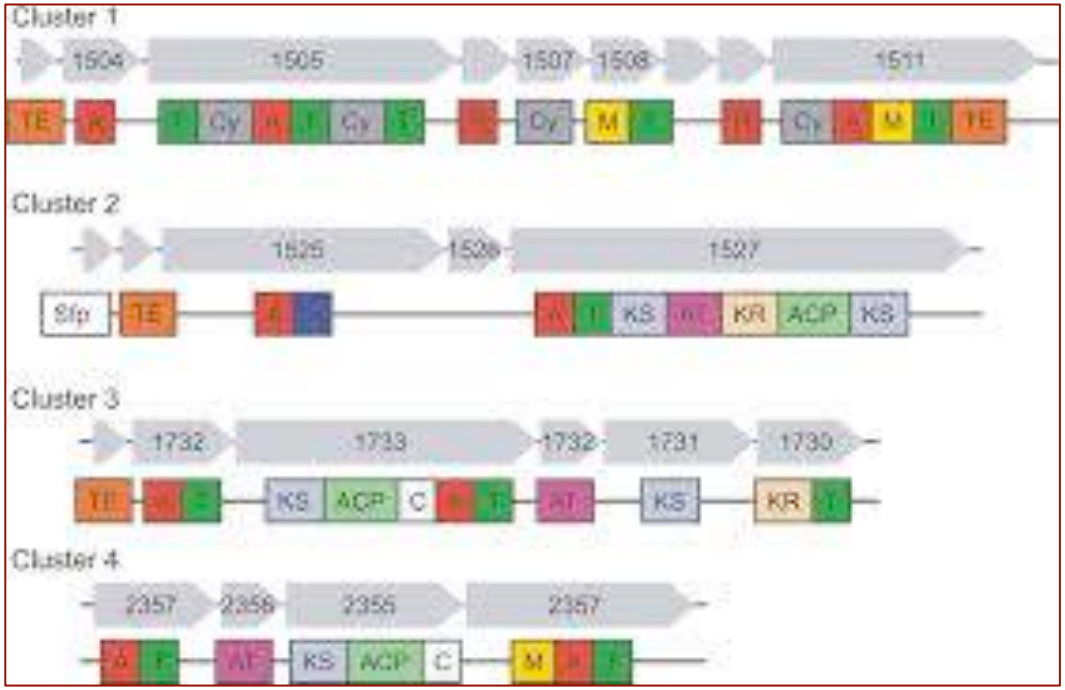
Endophenazine B

Mycometoxin A

Phenazine



SNS-32- G6-C



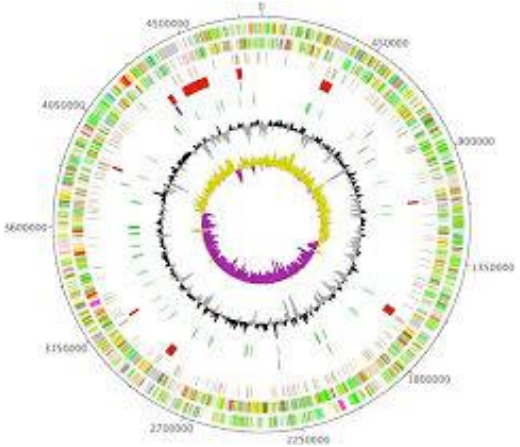
Polyketide - Polyketide synthases

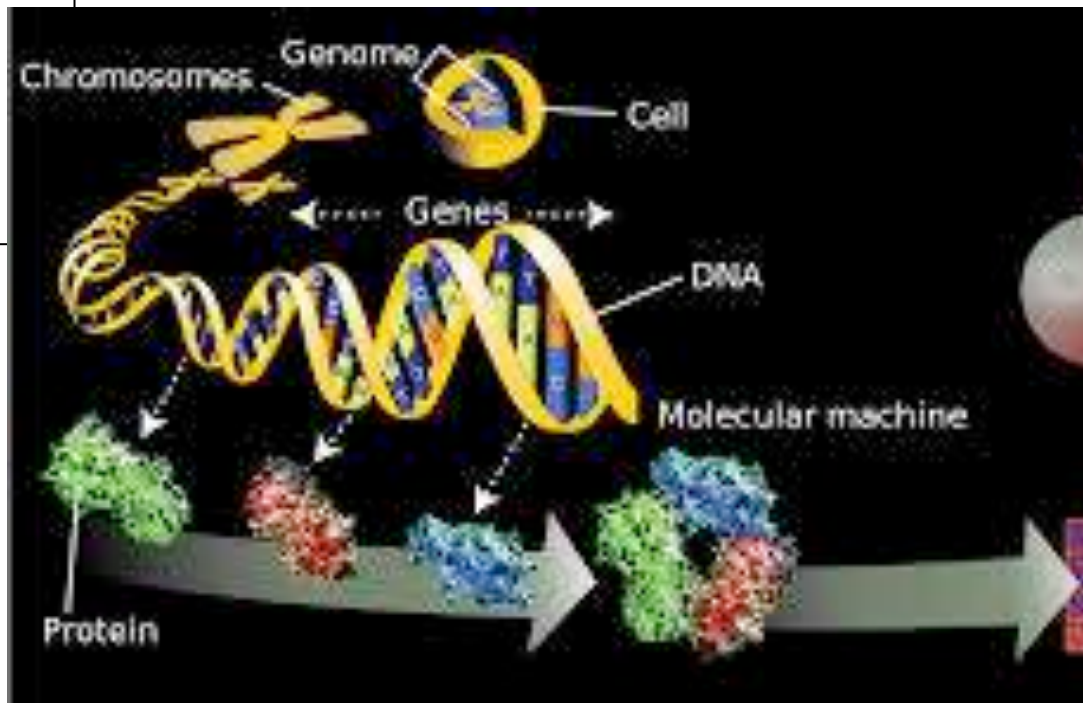
NRPS – Nonribosomal peptide

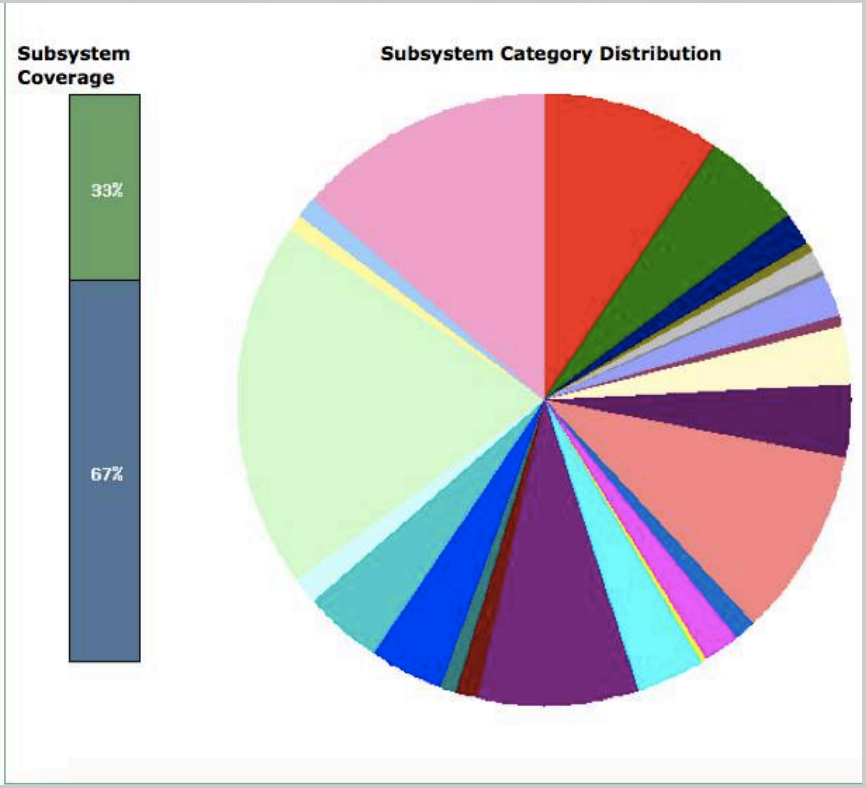
Genome Sequencing of Streptomyces sp. CMAA1322

Comparing expressed <u>metabolites</u> (Produced *in vitro*) and <u>gene clusters</u> (annotated in the genome)

- Metabolic Secondary presence;
- Clusters – PKS/NRPS;
- Pathway biosynthesis
- Biotechnological relevant compounds







Subsystem Feature Counts

- Cofactors, Vitamins, Prosthetic Groups, Pigments (307)
- Cell Wall and Capsule (161)
- Virulence, Disease and Defense (60)
- Potassium metabolism (15)
- Photosynthesis (0)
- Miscellaneous (33)
- Phages, Prophages, Transposable elements, Plasmids (12)
- Membrane Transport (67)
- Iron acquisition and metabolism (16)
- RNA Metabolism (98)
- Nucleosides and Nucleotides (126)
- Protein Metabolism (314)
- Cell Division and Cell Cycle (39)
- Motility and Chemotaxis (3)
- Regulation and Cell signaling (59)
- Secondary Metabolism (10)
- DNA Metabolism (120)
- Fatty Acids, Lipids, and Isoprenoids (271)
- Nitrogen Metabolism (35)
- Dormancy and Sporulation (26)
- Respiration (124)
- Stress Response (135)
- Metabolism of Aromatic Compounds (42)
- Amino Acids and Derivatives (613)
- Sulfur Metabolism (27)
- Phosphorus Metabolism (42)
- Carbohydrates (421)

Genome: 7,055,077 bp
Predicted to encode proteins:

- Stress responses;
- Osmotic stress,
- Heat shock, and
- Oxidative stress.

PacBio RS SMRT cell
(Pacific Biosciences BioProject)

Análise Antismash :

Cluster	Classificação
Cluster 1	Bacteriocina
Cluster 2	T1 PKS
Cluster 3	T2 PKS
Cluster 4	Phenazinas
Cluster 5	NRPS
Cluster 6	Indole
Cluster 7	Terpene
Cluster 8	other
Cluster 9	NRPS
Cluster 10	Bacteriocina
Cluster 11	Terpeno
Cluster 12	T1 PKS
Cluster 13	Lantipeptideo
Cluster 14	T1 PKS
Cluster 15	Other
Cluster 16	Terpene -T1PKS
Cluster 17	NRPS
Cluster 18	T2 PKS
Cluster 19	Terpeno
Cluster 20	Terpeno
Cluster 21	T3 PKS
Cluster 22	Terpeno
Cluster 23	Lantipeptideo
Cluster 24	T2 PKS
Cluster 25	Sideroforos
Cluster 26	T3 PKS
Cluster 27	T1 PKS
Cluster 28	T1PKS-NRPS
Cluster 29	T1PKS-T3PKS
Cluster 30	NRPS
Cluster 31	Sideroforos
Cluster 32	Terpeno
Cluster 33	T1 PKS
Cluster 34	T1 PKS
Cluster 35	NRPS-T1PKS
Cluster 36	NRPS-Terpeno
Cluster 37	T1 PKS
Cluster 38	Sideroforos
Cluster 39	T1 PKS
Cluster 40	T2 PKS
Cluster 41	NRPS-T1PKS

Obrigada...

- **PERGUNTAS**??
- suikinai@gmail.com