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Extremophiles from unique ecosystems of Kazakhstan as potential producers of novel antibiotics

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Ob' R.
Yenisey R.
Irtysh R.
Russia
Tura R.
Kama
Volga R.
Belaya R.
Petropavl
Qostanay
Rüdnyy
Ishim R.
Tobol R.
Oral
Chapaev
Aqtöbe
Astana
Ekibastuz
Semey
Oskemen
Oktyabr'
Temirtau
Qaraghandy
Ozero Zaysan
Ural R.
Emba
Kazakhstan
Ayaguz
Atyrau
Chelkar
Ozero Alakol'
Aral
Balqash
Beyneu
Saryshagan
Lake Balkhash
Aral Sea
Leninsk (Tyuratam)
Aqtaü
Qyzylorda
Ile R.
Khorogos
Syr Dar'ya R.
Shü
Almaty
Türkistan
Azer.
Baku
Shymkent
Bishkek (Frunze)
Yarkant R.
Uzbekistan
Tashkent
Kyrg.
Caspian Sea
Turkmenistan
China
Amu Dar'ya R.
Tajikistan
Ashgabat
Dushanbe
Iran
Tehran
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Institute of Microbiology and Virology Almaty, Kazakhstan

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Institute of Microbiology & Virology, Almaty, Kazakhstan

- Institute of Microbiology and Virology was founded in 1956. At present it is a leader research Institute in Kazakhstan in the field of basic and applied microbiology and virology
- Some research focus area include the investigations of microorganisms biodiversity in different ecosystems of Kazakhstan, maintenance and replenishment of collection of microorganisms, search and study of novel antibiotics, improvement of activity of antibiotic production, and the isolation and studying of biological, antigenic and molecular properties of new virus strains

Lead Extremophile Collaborator (since 2006)
Dr. Lyudmila Trezhnovikova

Virology Collaborator (since 2011)
Dr. Vladimir Berezin

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Outline

- Background
  - Antibiotics and resistance
  - Drug Discovery and Natural Products
- Study: goals, approach (screening) and findings
  - IMV study
  - IMV-USF collaboration (pilot study)
- Preliminary chemical characterization
- Future direction

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Table 12.1 Characteristics of the Ideal Antimicrobial Drug

- Selectively toxic to the microbe but nontoxic to host cells
- Microbicidal rather than microbistatic
- Relatively soluble; functions even when highly diluted in body fluids
- Remains potent long enough to act and is not broken down or excreted prematurely
- Doesn't lead to the development of antimicrobial resistance
- Complements or assists the activities of the host's defenses
- Remains active in tissues and body fluids
- Readily delivered to the site of infection
- Reasonably priced
- Does not disrupt the host's health by causing allergies or predisposing the host to other infections

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Table 12.2 Terminology of Chemotherapy

Chemotherapeutic Drug	Any chemical used in the treatment, relief, or prophylaxis of a disease
Prophylaxis	Use of a drug to prevent imminent infection of a person at risk
Antimicrobial Chemotherapy	The use of chemotherapeutic drugs to control infection
Antimicrobials	All-inclusive term for any antimicrobial drug, regardless of its origin
Antibiotics	Substances produced by the natural metabolic processes of some microorganisms that can inhibit or destroy other microorganisms
Semisynthetic Drugs	Drugs that are chemically modified in the laboratory after being isolated from natural sources
Synthetic Drugs	Drugs produced entirely by chemical reactions
Narrow Spectrum (Limited Spectrum)	Antimicrobials effective against a limited array of microbial types—for example, a drug effective mainly on gram-positive bacteria
Broad Spectrum (Extended Spectrum)	Antimicrobials effective against a wide variety of microbial types—for example, a drug effective against both gram-positive and gram-negative bacteria

Targets of Antimicrobials

1. Cell wall inhibitors

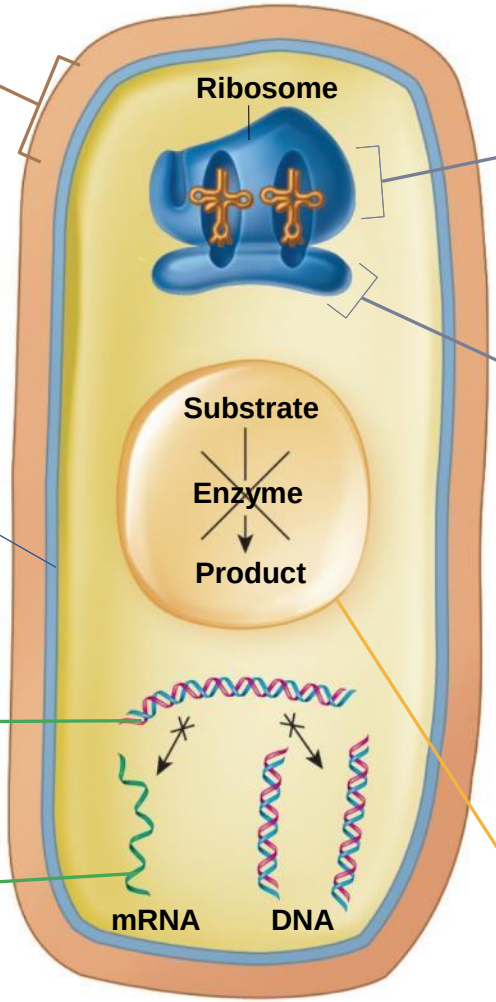
Block synthesis and repair
- Penicillins
- Cephalosporins
- Carbapenems
- Vancomycin
- Bacitracin
- Fosfomycin
- Isoniazid

2. Cell membrane

Causelossofselective permeability
- Polymyxins
- Daptomycin

3. DNA/RNA

Inhibit replication and transcription
- Inhibit gyrase(unwinding enzyme)
  - Quinolones
- Inhibit RNA polymerase
  - Rifampin



4. Protein synthes is inhibitors acting on ribosomes

Site of action 50S subunit
- Erythromycin
- Clindamycin
- Synercid
- Pleuromutilins

Site of action 30S subunit
- Aminoglycosides
  - Gentamicin
  - Streptomycin
- Tetracyclines
- Glycylcyclines

Both 30S and 50S

Blocks initiation of protein synthesis
- Linezolid

5. Folic acid synthesis

Block pathways and inhibit metabolism
- Sulfonamides (sulfa drugs)
- Trimethoprim

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Major Antimicrobial Drug Groups

- About 260 different antimicrobial drugs
- Classified in 20 drug families
- Largest number of antimicrobial drugs are for bacterial infections
  - Antibiotic Source:
    - fungi and bacteria
    - semi-synthetic compounds

Spectrum of Activity

Table 12.3 Spectrum of Activity for Antibiotics

Bacteria	Mycobacteria	Gram-negative Bacteria	Gram-positive Bacteria	Chlamydias	Rickettsias
Examples of diseases	Tuberculosis	Salmonellosis, plague, gonorrhea	Strep throat, staph infections*	Chlamydia, trachoma	Rocky Mountain spotted fever
Spectrum of activity of various antibiotics	Isoniazid	Streptomycin, Tobramycin, Polymyxin, Carbapenems, Tetracyclines, Sulfonamides, Cephalosporins, Penicillins			
Are there normal biota in this group?	Yes	Yes	Yes	Probably	None known

*Note that some members of a bacterial group may not be affected by the antibiotics indicated, due to acquired or natural resistance. In other words, exceptions do exist.

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Aminoglycoside Drugs

- Products of various species of soil actinomycetes in the genera *Streptomyces* and *Micromonospora*
- Relatively broad spectrum because they inhibit protein synthesis
- Subgroups and uses
  - Aerobic gram-negative rods and certain gram-positive bacteria
  - Streptomycin: Bubonic plague and tularemia and good anti-tuberculosis agent
  - Gentamicin: Less toxic and used for gram-negative rods

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Resistance Mechanisms

- Antibiotics are present in nature
- Microbes are capable of adapting quickly to selective pressures
- Drug resistance has arisen for all antibiotics
- ESKAPE pathogens
- Two main strategies employed by microbes
  - Prevent access of the drug to the target site
  - Alter the nature of the target site

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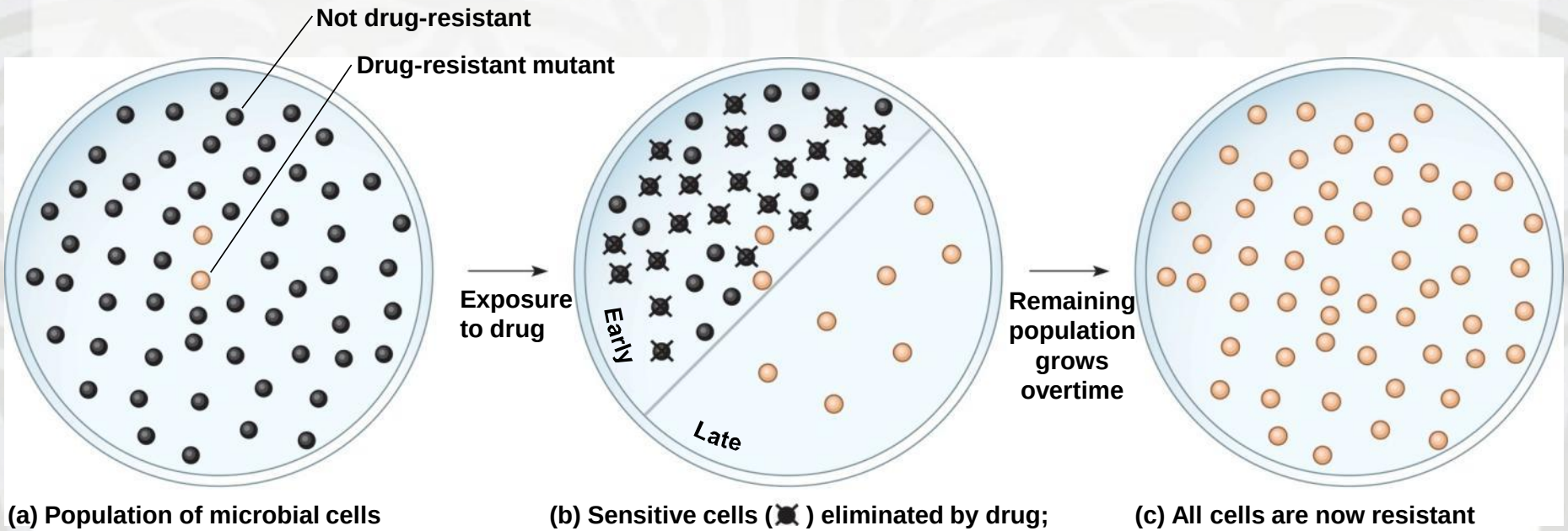
Antibiotic Resistance



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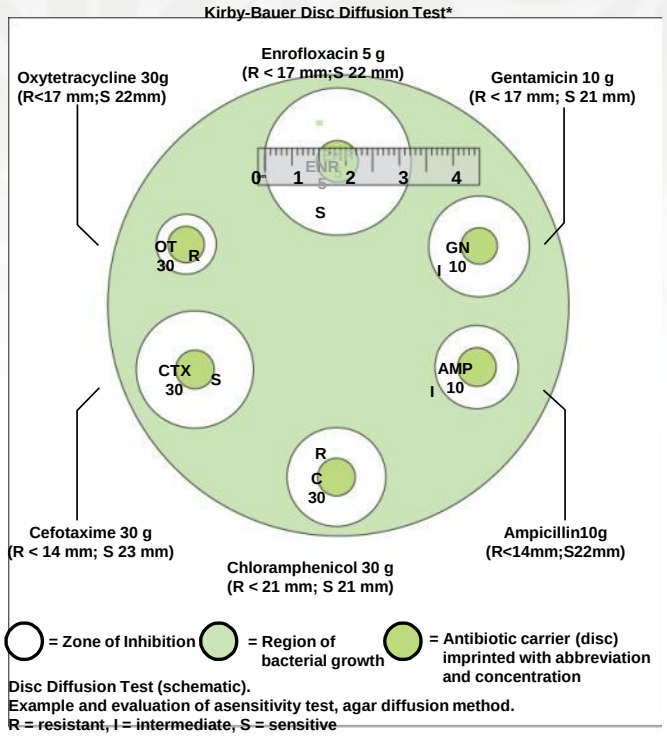
Disk Diffusion Assays

- Kirby-Bauer
- Standardized conditions
- Zones of inhibition
- Larger zone indicates more susceptible
- Smaller zone indicates more resistant



b: © Kathy Park Talaro

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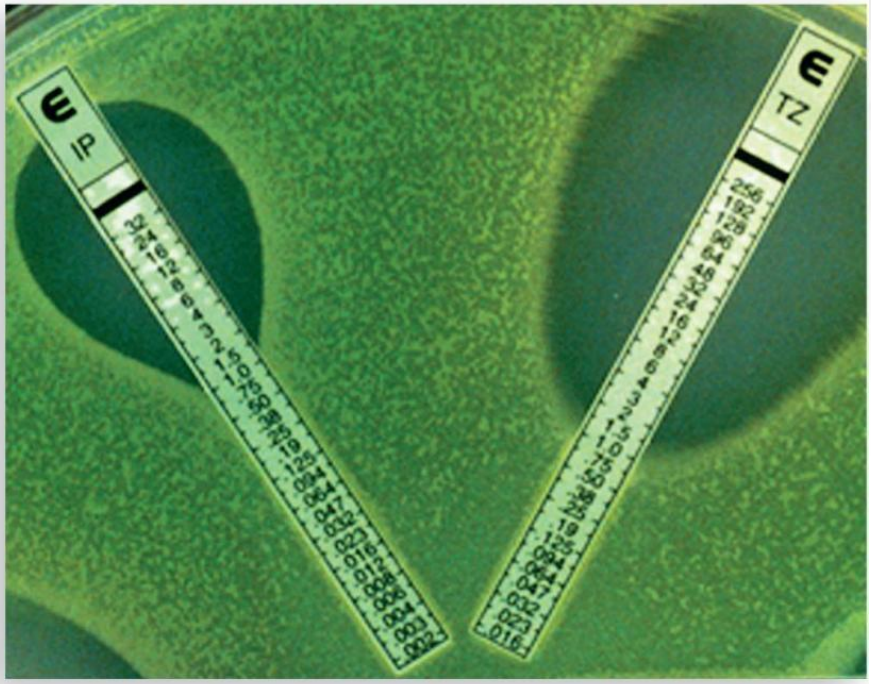
E-Test Strips

- Drug gradient used
- Can determine MIC
- Read where the zone touches the strip



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DRUG DISCOVERY AND NATURAL PRODUCTS

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Starting point

Research and development of drug or vaccines products is

- demanding
- risky

How are they valued?

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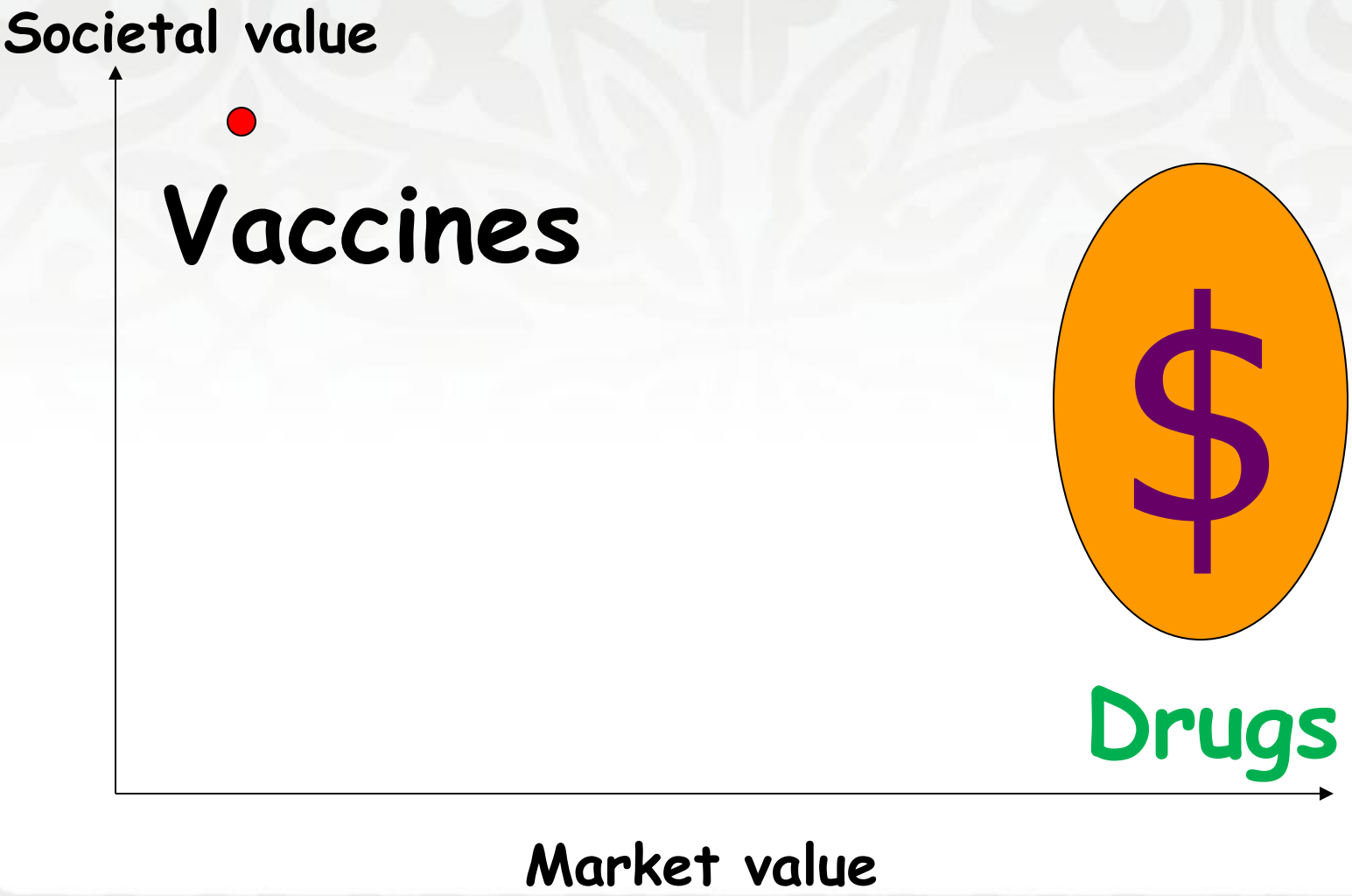

The path toward a drug

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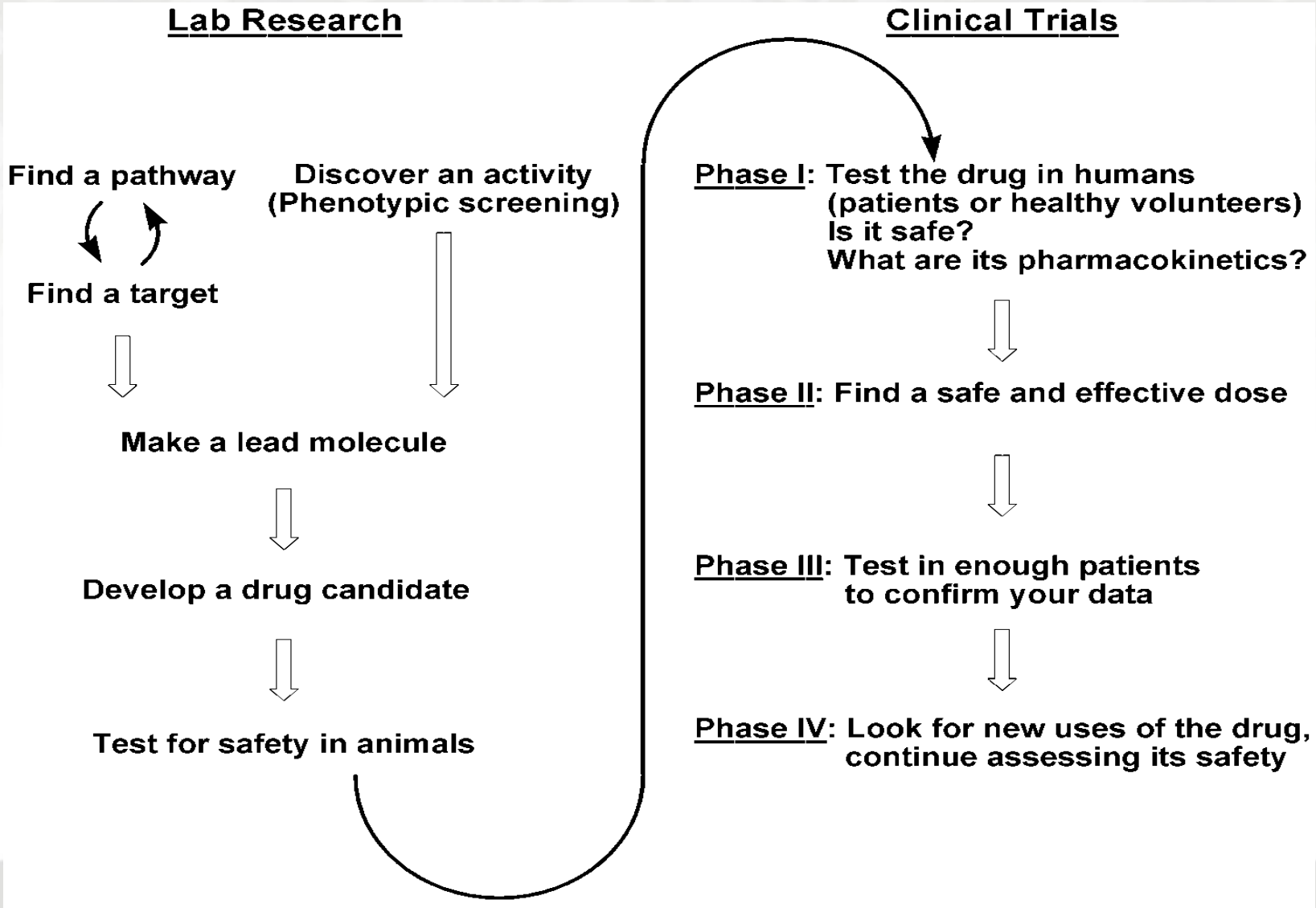


Cautious Optimism for the Antibacterial Pipeline

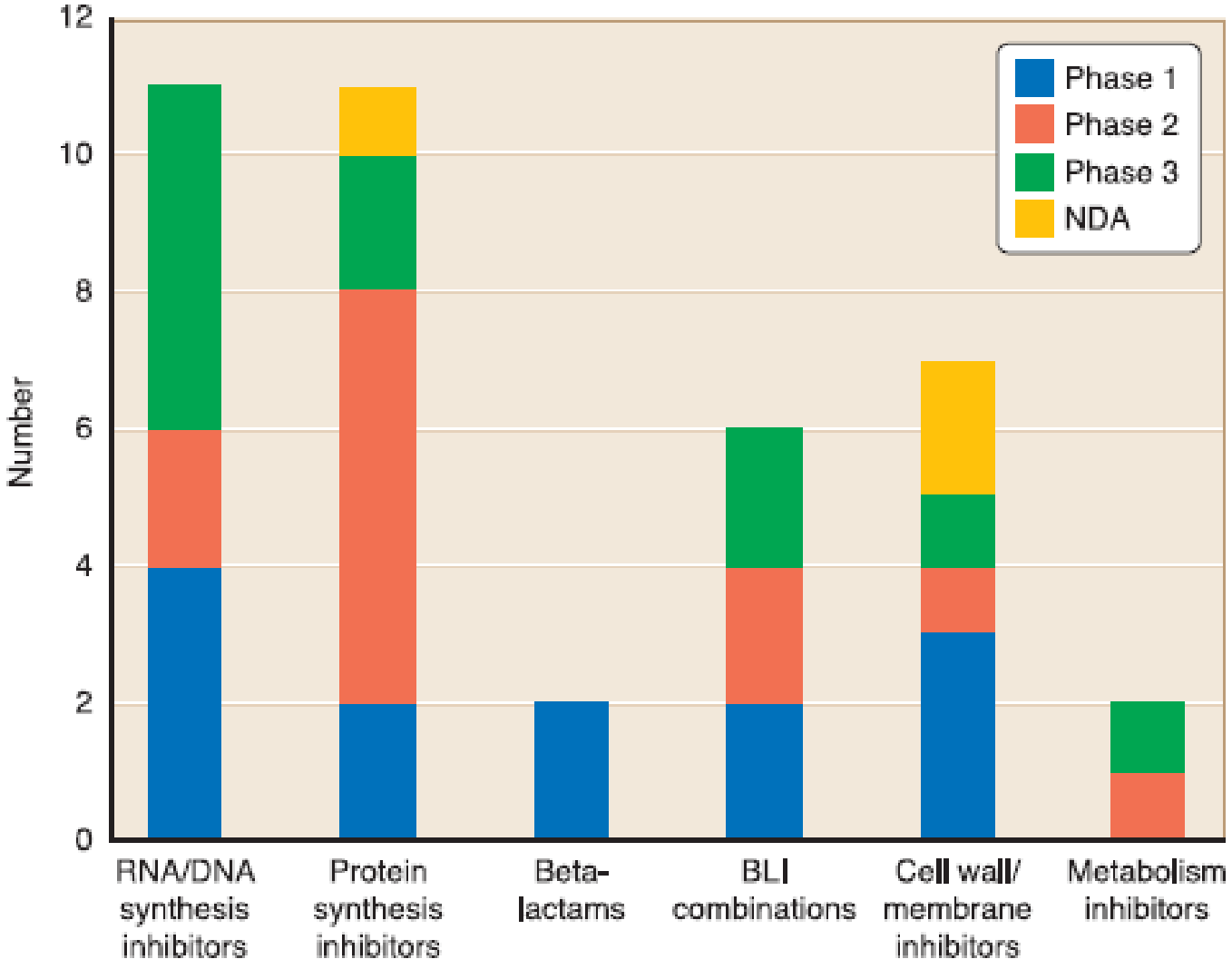
Microbe—Volume 9, Number 4, 2014

Despite dire warnings, there is resurgent effort to develop novel antibiotics, with several dozen candidate drugs already entered into clinical trials

Michael J. Pucci, Malcolm G. P. Page, and Karen Bush

- **In spite of pessimism; there are many antibacterial agents in clinical trials**
  - Novel agents on the rise: last 85 years
  - Gram positive, gram negative and multi-drug resistant
- 39 antibacterial compounds
  - 25 in Phase 2 or Phase 3 Trials
- "Guarded optimism"
  - Renewed commitment from companies
  - Cubist (acquired Optimer and Trius Therapeutics), Roche

FIGURE 2



The number of investigational agents in clinical development according to mechanism of action.

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Natural Products

- Natural products originate from bacteria, fungus, plants or other natural sources (marine organisms)
- Scaffold for many effective antibiotics (semi-synthetic)
- Screening natural products is complicated
  - Complex mixture of secondary metabolites
  - Rediscovery of known active compounds
    - Dereplication to rule out knowns
    - Competition with HTS of synthesized compounds
  - Competition with HTS of synthesized compounds
  - Purified natural compounds exert challenge

- Success (using *in-vitro* bioassay) depends on
  - Proper design, validation and implementation of screening assays

Planta Med 2014; 80: 1182–1199

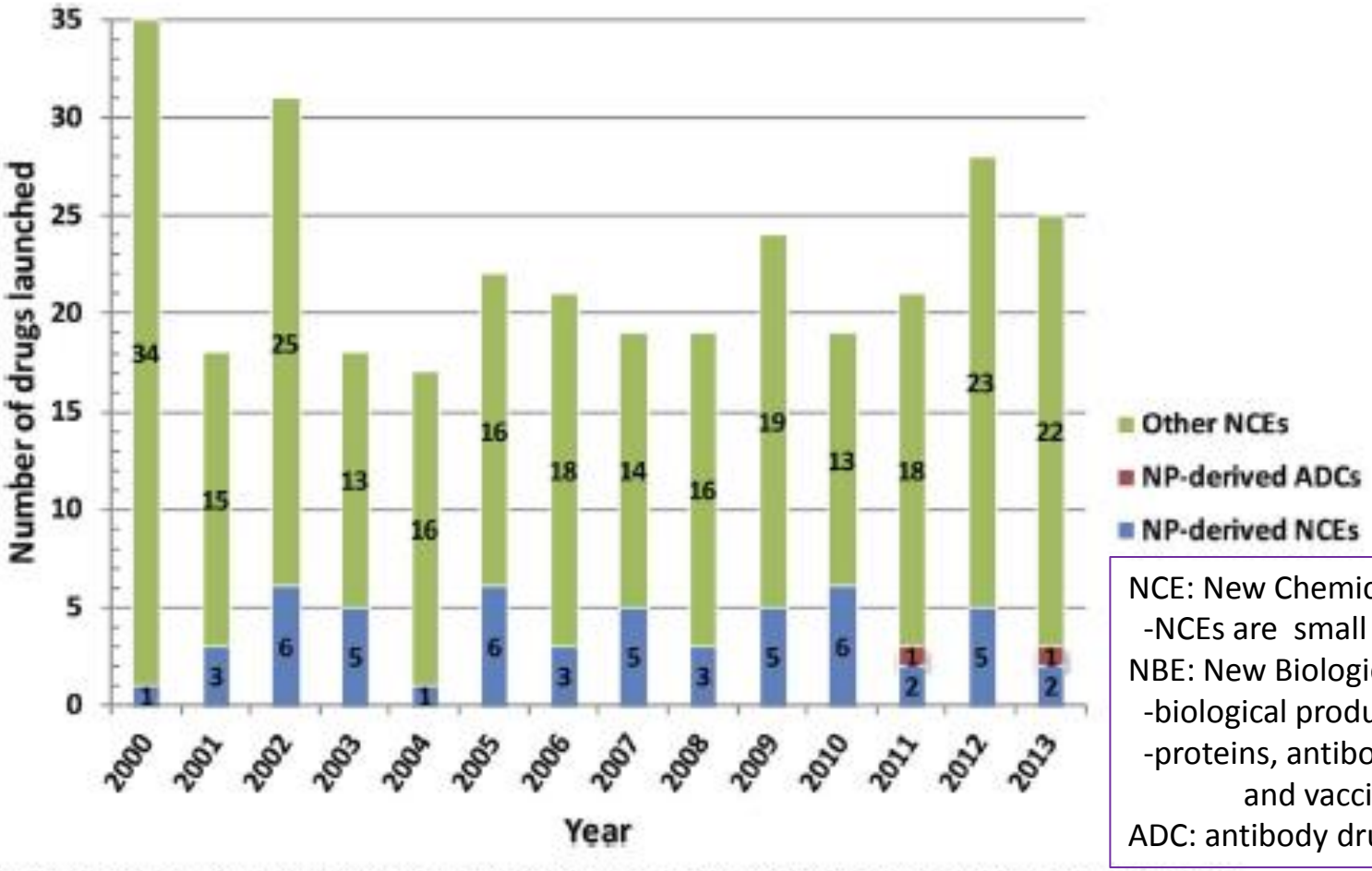
The re-emergence of natural products for drug discovery in the genomics era

Alan L. Harvey[1,2], RuAngelie Edrada-Ebel[2] and Ronald J. Quinn[3]

NATURE REVIEWS | DRUG DISCOVERY

VOLUME 14 | FEBRUARY 2015 | 111

- Natural products (NP) are a rich source of compounds for drug discovery (34%: 1981-2010)
- Their use has decreased in the last 2 decades; barriers: NP screening in HTS against Targets
- Review: technical advances to reduce barriers
  - Genomic and metabolomic approaches
  - Augment traditional methods of screening
  - Increased functional assays and phenotypic screen



NCE: New Chemical entities
-NCEs are small molecules
NBE: New Biological entities
-biological products :
-proteins, antibodies, viruses and vaccines.
ADC: antibody drug conjugates

Fig. 1 Worldwide NCEs approved from 2000–2013 divided into NP-derived NCEs, NP-derived ADCs and other NCEs.

Natural product and natural product derived drugs in clinical trials†

Mark S. Butler,* Avril A. B. Robertson and Matthew A. Cooper

| *Nat. Prod. Rep.*, 2014, **31**, 1612–1661

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BACKGROUND

- Bacteria in the order Actinomycetales account for 45% of the bioactive microbial metabolites discovered
- These organisms have played a central role in the development of the modern pharmaceutical industry.
- The search for novel antibiotics for use in medicine – important aspect of soil microbial diversity.

Objective of study:

1) Collect soil samples from extreme environments of Kazakhstan
2) Isolate & characterize extremophiles as potential producers of novel antibiotics

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Kazakhstan Extremophiles

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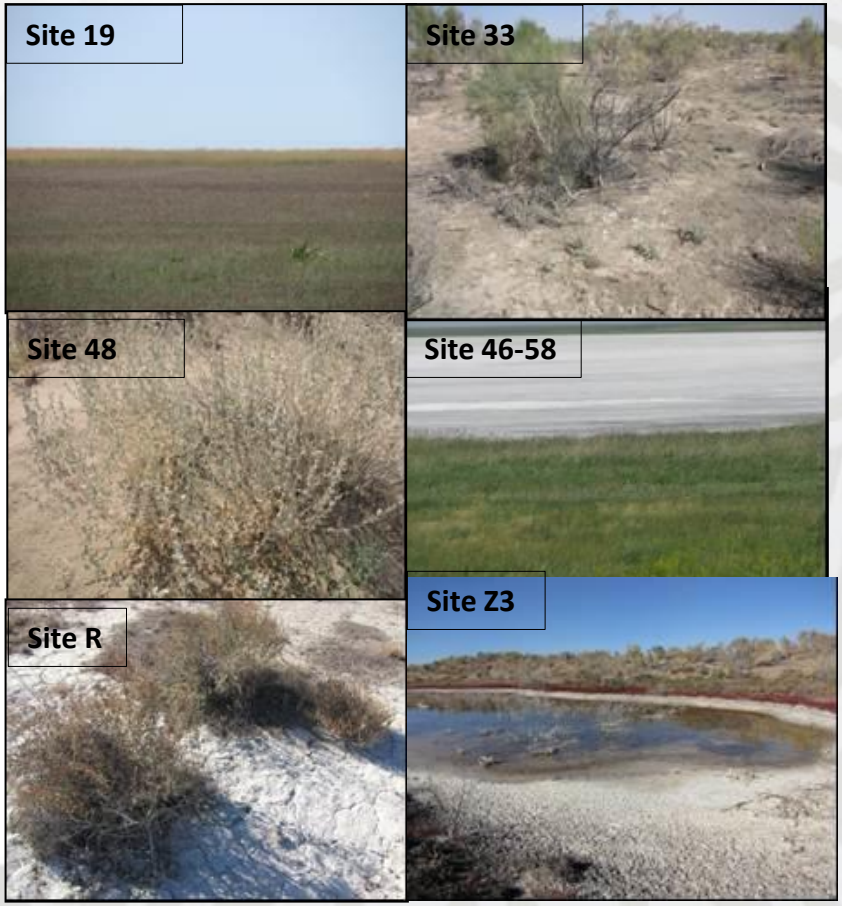
The purpose of this project is the study of biodiversity of extremophiles and screening of microorganism strains with the industrially valuable properties from the soils of Kazakhstan.

Collections and Selection of samples for analyses

1. The collection of soil and mud samples from extreme geographical zones of Kazakhstan (solonchak, solonets, and solod soils, mineral water sources, anthropogenic area).
2. Research of microorganism diversity in the extreme habitats (natural and anthropogenic).
3. Isolation of pure strains of extremophiles from soils and muds.
4. Study of antimicrobial activity of the extremophiles against gram-positive and gram-negative test-organisms, including clinical resistant strains.
5. Selection of strains with the potential for industrial application and their id with PCR
6. Creating a collection of extremophiles as producers of new industrially valuable biologically active substances.

Some snap-shots of sites (North and South) in Kazakhstan



Methods and findings

Sampling of Soil

- 5,936 soil samples from Kazakhstan soil and marine environments

Isolation of Actinomycetes

- 2,019 isolates, morphologically consistent with actinomycetes were grown on 3 variants of Bennett's agar

Extraction of antibiotic from cultures

- Soil samples were plated following the dilution plating method on modified Bennett's agar with the following contents: glucose – 0.2%, peptone – 0.2%, yeast extract -0.1%, agar -2%, pH 7.2.
- Actinomycetes from the natural substrate samples were isolated on the three variants of modified Bennett's agar:
  - #1 – modified Bennett's agar, pH 7.2;
  - #2 - modified Bennett's agar +5% NaCl, pH 7.2;
  - #3 - modified Bennett's agar +0.5% Na2CO3, pH 9.0.
- Bacterial strains: hospital strain MRSA # 3316 and *Escherichia coli* (pMG223) were used in this study.
- Actinomycetes isolates screened through 2 stages; disc diffusion method according to Barry, A.L. and C. Thornsberry*

Shipped to the US, Screening

Kirby-Bauer Protocol (USF)

CDDI (USF) Chemical Analyses

* Barry, A.L. and C. Thornsberry, 1985. Susceptibility Tests: Diffusion test procedure. In: Manual of Clinical Microbiology, 4th Edn., Ballows, E.A., W.J. Hawsler Jr and H.I. Shadomy (Eds.). American Society of Microbiology, Washington D C., pp: 978-987.

Primary Screening: disc diffusion

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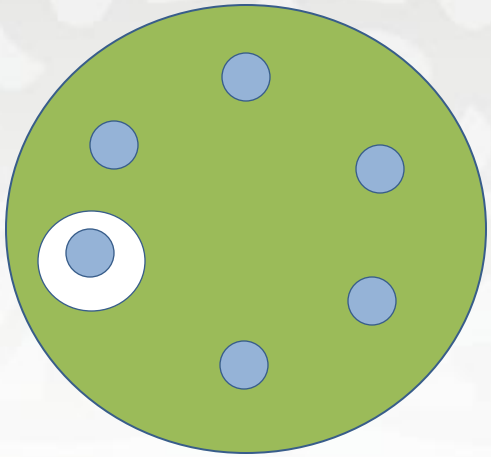
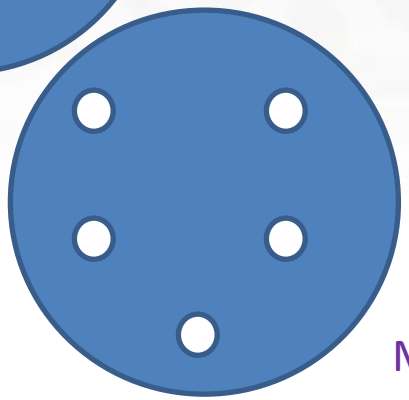
Media #1

Crude screening: use cork-borer to transfer Actinomycetes strains to lawn of test organisms





Media #2…etc

1) Actinomycetes from the natural substrate samples were isolated on the three variants of modified Bennett's agar:
   #1 – modified Bennett's agar, pH 7.2;
   #2 - modified Bennett's agar +5% NaCl, pH 7.2;
   #3 - modified Bennett's agar +0.5% Na_2CO_3, pH 9.0.
2) Pure culture was obtained and a lawn of bacteria was prepared
3) Using cork-borer, the pure culture was transferred to a lawn of test organism (*E.coli* and *S. aureus*)

1) Pure culture of test organism grown as a lawn
2) MRSA # 3316 and *Escherichia coli* (pMG223)
3) Zone of inhibition noted
4) Extremophile culture condition that produces antagonistic activities determined

Extraction - crude (not pure compound)…

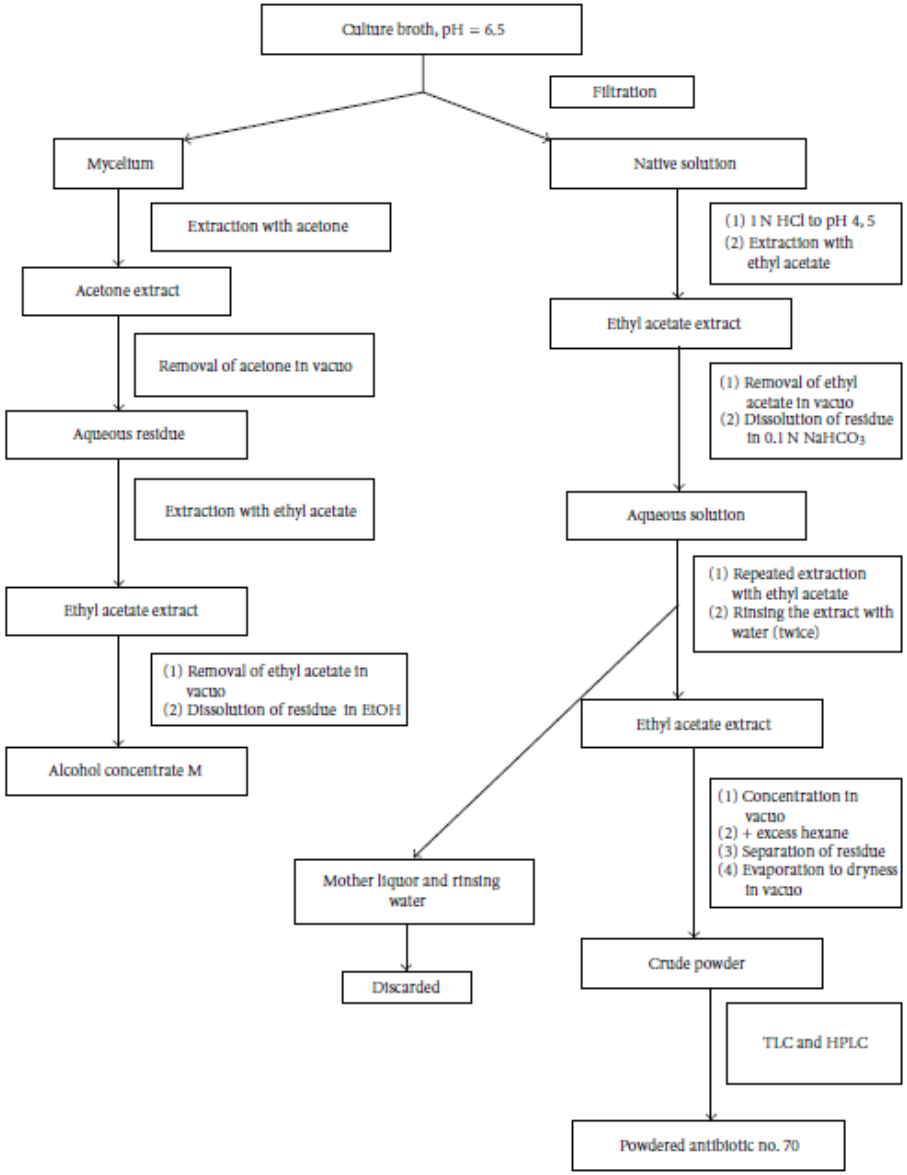


Figure 1: Flowchart for isolation of antibiotic no. 70. Antibiotic no. 70 was isolated from culture broth of the producer IMV-70 by extraction methods. The flowchart for the extraction that produces the powder preparation of compound no. 70 is shown in Figure 1. The antibiotic no. 70 was isolated from culture broth by extraction with ethyl acetate, was subsequently purified from the inactive lipid fraction, and was extracted from the concentrated solution with hexane.

Crude Extraction

The Scientific World Journal
Volume 2012, Article ID 594231, 8 pages
doi:10.1100/2012/594231

The ScientificWorldJOURNAL

Research Article

Characterization of the Antibiotic Compound No. 70 Produced by *Streptomyces* sp. IMV-70

Lyudmila P. Trenozhnikova,[1] Almagul K. Khasenova,[1] Assya S. Balgimbaeva,[1] Galina B. Fedorova,[2] Genrikh S. Katrukha,[2] Nina L. Tokareva,[2] Boo H. Kwa,[3] and Azliyati Azizan[3]

[1] *Institute of Microbiology and Virology, Ministry of Education and Science Committee, 103, Bogenbay batyr Street, Almaty, Kazakhstan*
[2] *Research Institute of New Antibiotics, Russian Academy of Medical Sciences, Moscow, Russia*
[3] *Global Health Department, College of Public Health, 13201 Bruce B. Downs Boulevard, Tampa, FL 33612, USA*

Secondary Screening: disc diffusion

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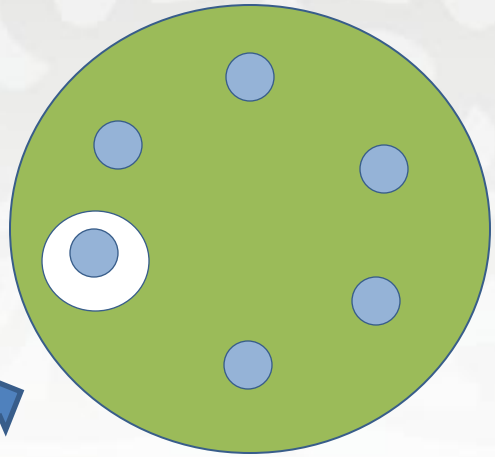
Crude Extracts Reconstituted

Sterile discs



1) Pure culture of test organism grown as a lawn
2) MRSA (KZ) and *Escherichia coli* (KZ), and MRSA (USA) and *Acinetobacter baumannii* (USA)
3) Sterile discs containing known concentrations of extracts overlaid on the lawn
4) Zone of inhibition noted
5) Extracts (and concentrations) that produce antagonistic activities determined

1) Crude extracts (powder) was reconstituted to various concentrations
2) These are applied in an aseptic manner to a set of sterile discs
3) Dried discs are then placed on the lawn of test organisms

Chemical characterization, etc

Microbiological characterizations

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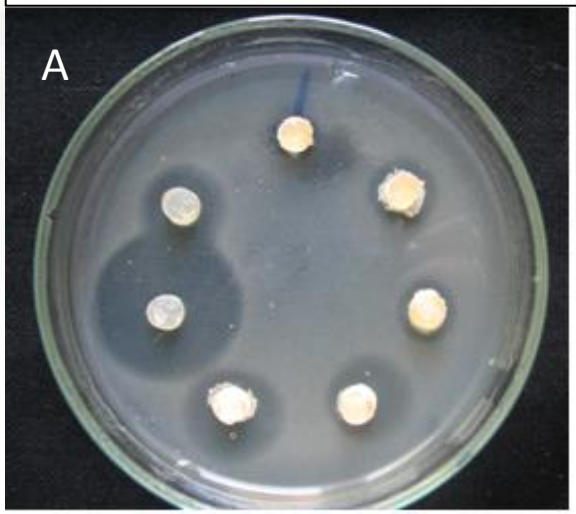
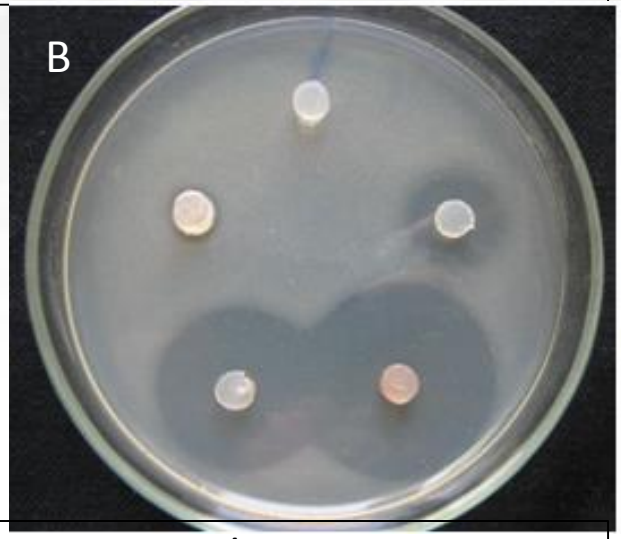
Fig 1. **Antagonistic properties of extremophile actinomycetes in high salt media, varying pH conditions against Gram positive organisms**





A. Extracts from Strain 91/1 against G+ pathogen
Top – growth on medium 1, pH 7; then clockwise (medium 1, pH 9), (medium 1 + 0,25% Na_2CO_3), (medium 1 + 0,375% Na_2CO_3), (medium 1 + 0,5% Na_2CO_3), (medium 1 + 0,75% Na_2CO_3), (medium 1 + 1% Na_2CO_3). Test-microorganism – *S.aureus* 209P, nutrient agar.

B. Extracts Strain 46/15 against G+ pathogens
Top – growth on salt-free medium (medium 1, pH 7), then clockwise (medium 1, pH 9), (medium 1 + 5% NaCl), (medium 1 + 7.5% NaCl), (medium 1 + 10% NaCl). Test-microorganism – *S.aureus* 209P, nutrient agar.

Subgroups of actinomycetes

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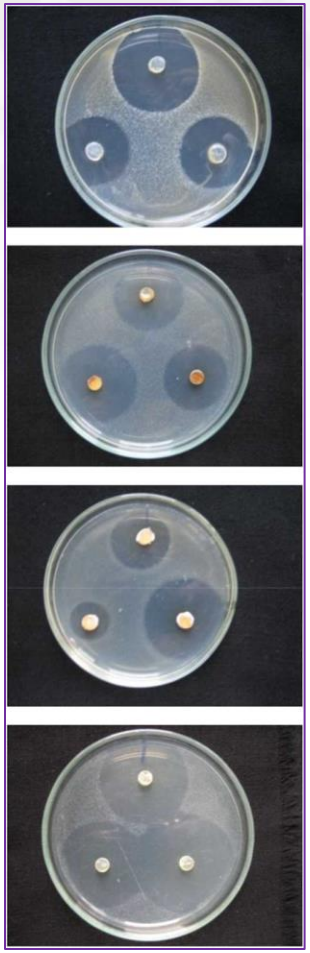
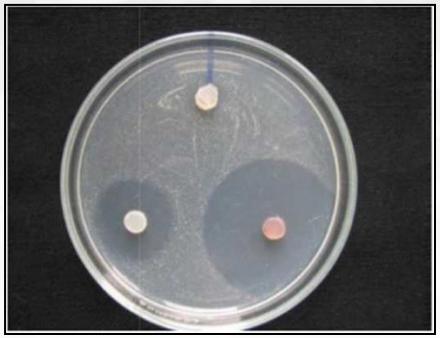
Group IA



Subgroup IBa

Subgroup IBc



Subgroup ICa

Subgroup IBb

Subgroup ICc

Subgroup ICb

Classification of actinomycetes based on ability to show antagonism in different habitats

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Subgroup	Antagonism in neutral habitat	Antagonism in saline habitat	Antagonism in alkaline habitat
IA	+	+	+

Classification of actinomycetes based on ability to show antagonism in different habitats

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Subgroup	Antagonism in neutral habitat	Antagonism in saline habitat	Antagonism in alkaline habitat
IA	+	+	+
IBa	+	+	-
IBb	+	-	+
IBc	-	+	+
ICa	+	-	-
ICb	-	+	-
ICc	-	-	+

Classification of actinomycetes based on ability to show antagonism in different habitats

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Subgroup	Antagonism in neutral habitat	Antagonism in saline habitat	Antagonism in alkaline habitat
IA	+	+	+
IBa	+	+	-
IBb	+	-	+
IBc	-	+	+
ICa	+	-	-
ICb	-	+	-
ICc	-	-	+
IIAa	+	+	**no growth**
IIAb	+	**no growth**	+
IIAc	**no growth**	+	+
IIBa	+	-	**no growth**
IIBb	-	**no growth**	+
IIBc	**no growth**	+	-

Streptomyces morphogenesis

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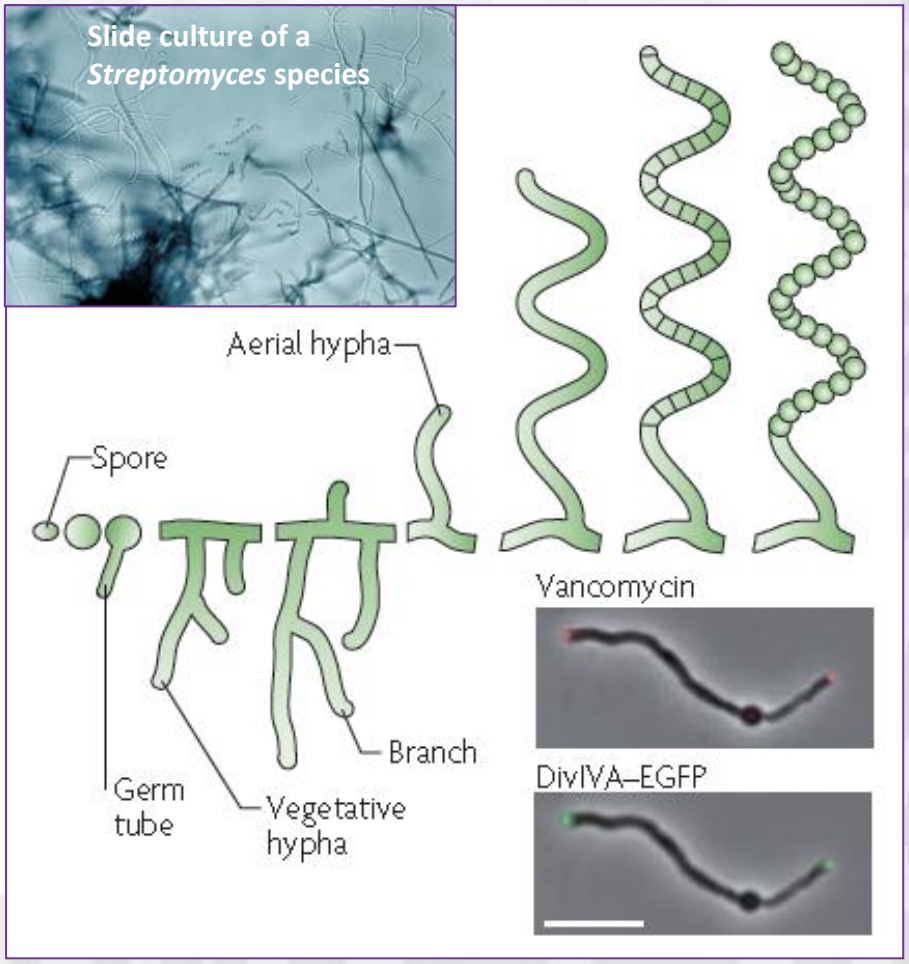
- *Streptomyces* largest genus of Actinobacteria, Order Actinomycetales and the type genus of family Streptomycetaceae
- Gram positive bacilli, about 550 species
- Found in soil and decaying vegetation
- Spores, hyphae, mycelium
- Regulatory genes: *afsB, bldA, whiG*
- Diverse secondary metabolites
  - important role in life cycle in nature
- Produce over 2/3 of the clinically useful antibiotics of natural origin
  - Chloramphenicol (from *S. venezuelae*)
  - Daptomycin (from *S. roseosporus*)
  - Neomycin (from *S. fradiae*)
  - Puromycin (from *S. alboniger*)
  - Streptomycin (from *S. griseus*)
  - Tetracycline (from *S. rimosus* and *S. aureofaciens*)
- Antifungal of medicinal importance
  - nystatin (from *S. noursei*),
  - amphotericin B (from *S. nodosus*)

Developmental life cycle of *Streptomyces coelicolor*



Microbiology (1996), 142, 1335–1344 Printed in Great Britain

SGM SPECIAL LECTURE

The regulation of antibiotic production in *Streptomyces coelicolor* A3(2)

Mervyn Bibb

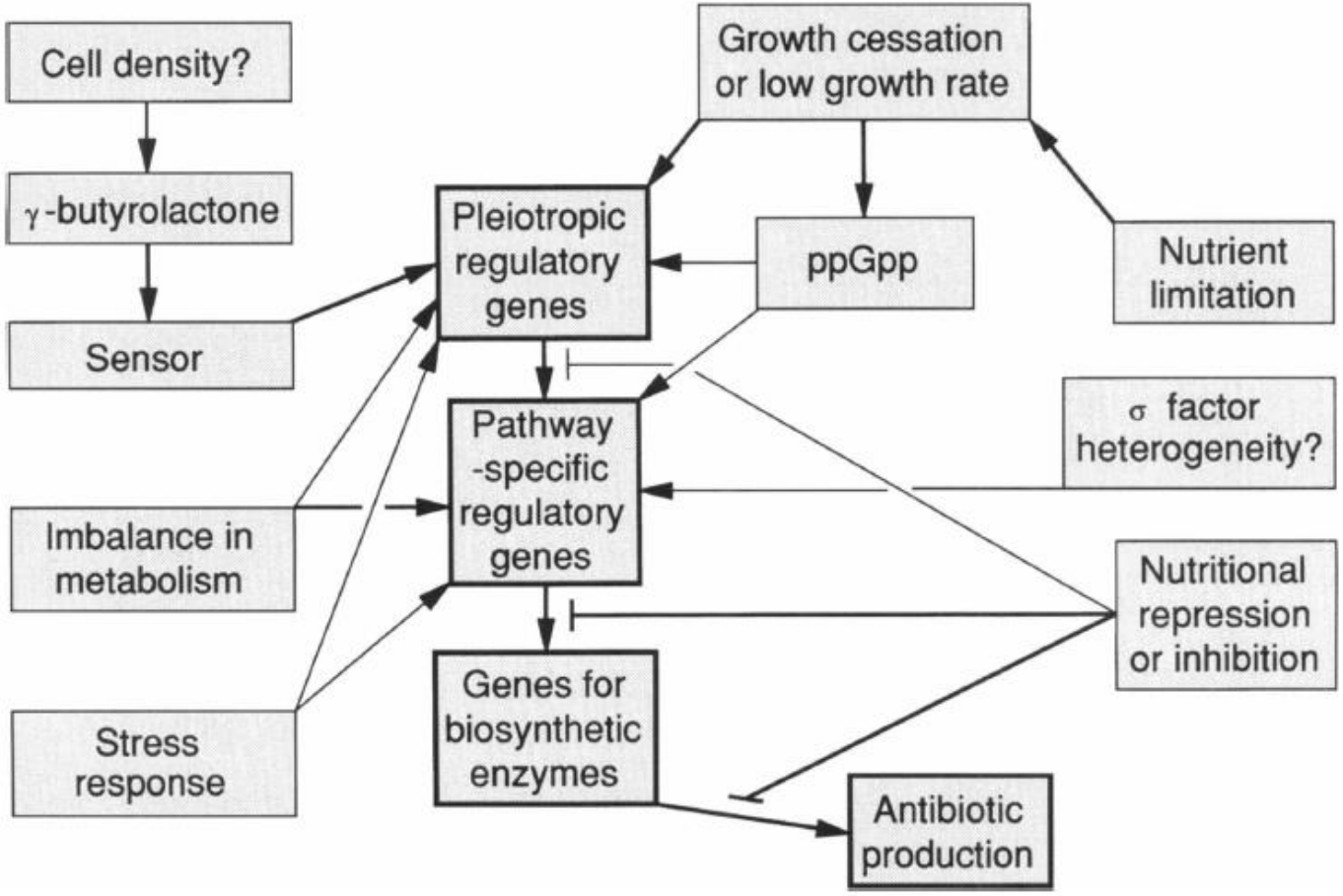


Fig. 3. Factors potentially determining the onset of antibiotic production in streptomycetes. Thinner lines represent plausible interactions for which there is currently no direct evidence.

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Results

- Actinomycetes strains analyzed based on their ability to show antagonism in the conditions of saline or alkaline environment;
- 415 strains with antagonistic properties were selected:
  - antagonism against clinical *MRSA* strains (100%):
  - 21.6% had activities against *E. coli*
  - 28.4% against *A. niger*.

- Changes in growth, morphogenesis, and antagonism of 415 strains of extremophile actinomycetes were determined in the habitats: neutral, saline, and alkaline.
  - The actinomycetes were classified into groups, subgroups, and variants.
  - The "cosmopolitan" (55.4%) dominated: grow and show antagonism in all studied habitats.

- Two variants of actinomycetes whose antagonism is inversely related to their morphogenesis were determined.
  - The variant Q “quitters” (39.8%) antagonism correlated to good growth and formation of aerial mycelium
  - The variant F “fighters” (60.2%) - antagonism correlated to the inhibition of growth and aerial mycelium.

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Pilot study
(IMV - USF Collaboration)

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Project Focus and Goals

Long term Focus of Collaboration

Characterization of extremophiles obtained through screening of the producers of valuable antibiotics from unusual (extreme) ecosystems of Kazakhstan

- The goal of the pilot project is to characterize a small sample of the extracts from Actinomycetes strains.
  - Compare susceptibility of extracts for antibiotics activity: Kazakhstan vs U.S. HAI pathogens (MRSA and *Acinetobacter*)
  - Chemical characterization and identification of putative active components (CDDI proteomics)

Timeline of collaboration & project Sample preparation & Analyses

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Grant from International Scientific and Technology Center, Russia (2006)

Institute of Microbiology and Virology (IMV), Republic of Kazakhstan (2006-2012)

(ECIA) Samples shipped to USF, USA (2011-2012)

Travel Grant to IMV (2011)

2012-2013: MRSA and Acinetobacter from Florida Hospital, USA

Disc Diffusion /proteomics analyses

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Background

- Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of multidrug resistant (MDR) hospital-associated infections (HAI) in the U.S
- MDR infections result in increased morbidity, mortality, cost of care, length of hospital stay, and increasingly insusceptible to known antimicrobials
- Multidrug-resistant (MDR) *Acinetobacter baumannii* is becoming an important healthcare- acquired pathogen in hospitals and other health care settings.
- *Acinetobacter baumannii* is an increasing cause of HAI in Intensive Care Units (ICUs) in the United States with it being the fifth most frequent cause of pneumonia
- Most antibiotics are natural products or semisynthetic derivatives from soil actinomycetes

Methods and findings

Sampling of Soil

Isolation of Actinomycetes

Extraction of antibiotic from cultures

- The antibiotic was fermented in two organic media and shaken for 96 hours
- Extracts were tested against *S. aureus* and *E. coli*
- Toxicity through *in-vivo* methods using inoculation into infected mice

Shipped to the US, Screening

- The dried preparations were selected for transport to USF
- Screened against U.S. hospital associated pathogens listed below, for antagonistic activities
- MRSA and *A. baumannii* from Florida Hospital were used for testing actinomycetes extracts

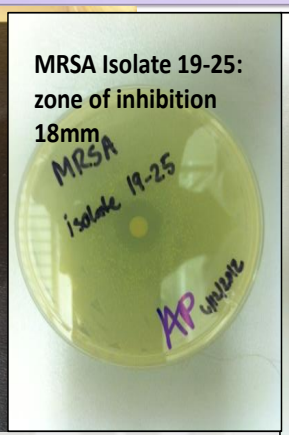
Kirby-Bauer Protocol (USF)

- MH agar was used to perform Kirby-Bauer antibiotic disk diffusion protocol
- Dried extracts were re-suspended in EtOH, applied to sterile discs, then prepared for the assay
- Disks with known antibiotics concentrations were used for positive and negative controls

CDDI (USF) Chemical Analyses

Fig. 3. Zone of inhibition of U.S. MRSA isolates against discs with KZ extracts

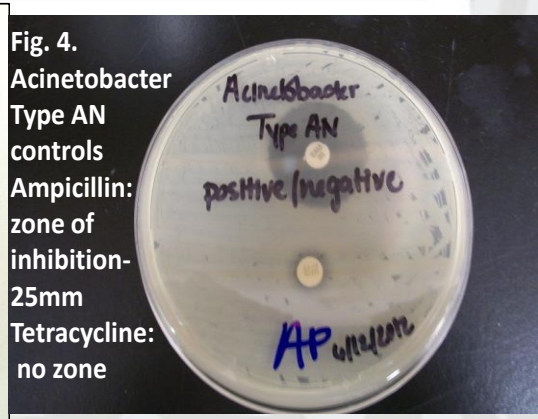
MRSA Controls
Sulfa/Trimethoprim : zone of inhibition-36mm
Tobramicin- no zone



MRSA Isolate 19-25: zone of inhibition 18mm

MRSA Isolate 48-29: zone of inhibition- 23mm

Fig. 4. Acinetobacter Type AN controls Ampicillin: zone of inhibition- 25mm Tetracycline: no zone



Overall summary of MRSA susceptibility to extremophile extracts

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Inhibition of Kazakhstan and US HA-MRSA by Actinomycetes Antagonists

Antagonist # and Source (pH)		Zone of Inhibition (Kazakhstan HA-MRSA) mm			United States HA-MRSA Zone of Inhibition[e]
		Growth Media			
		1[a]	2[b]	3[c]	
2-2	mud (9.1)	0	18	NG	0
6-12	rhizosphere (8.6)	0	25	NG	0
18-7	sandy soil (10.0)	13	44	NG	0
19-25	soil (9.3)	0	35	0	11.5
33-1	mud (9.6)	NG[d]	49	39	10.0
36-3	meadow soil (8.3)	10	24	23	0
41-8	saline soil (10.0)	11	29	15	7.0
48-29	sandy soil (10.0)	0	32	22	20.5
51-9	rhizosphere (9.5)	10	46	10	0
58-22	rhizosphere (8.9)	0	18	14	22.5
72-1	soil (9.6)	0	50	NG	0
96-1	soil (8.6)	11	26	19	0
Q4-39	soil (10.0)	0	16	15	0
Y-45	rhizosphere (9.8)	0	20	16	0

[a] Growth Medium 1 = Modified Bennett's pH=7.2
[b] Growth Medium 2 = Modified Bennett's pH=7.2, 5% NaCl
[c] Growth Medium 3 = Modified Bennett's pH=9.0, 0.5% Na_2CO_3
[d] NG = No growth of the Actinomycetes producer
[e] Zone of Inhibition for US HA-MRSA reported is an average of multiple DDAs.

Methods and findings

Sampling of Soil

Isolation of Actinomycetes

Extraction of antibiotic from cultures

Shipped to the US, Screening

Kirby-Bauer Protocol (USF)

CDDI (USF) Chemical Analyses

http://www.research.usf.edu/cddi/

- Detection and characterization of potential active molecules by Chemists at the USF CDDI proteomics facility using the analytical LC/DAD/MS instrumentation

USF Center for Drug Discovery and Innovation (CDDI)

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- **LCMS analysis summary**
- **Mass Spectrometry (MS):** Agilent 6120 single quadrupole
- **Ion Mode:** electrospray (**ESI**), positive and negative
- **Mass range acquisition:** m/z 100-1200 amu
- **Software:** chemstation

- **High Performance Liquid Chromatography (HPLC):** Agilent 1100 Binary Pump, Well Plate autosampler, (2mL vial) Diode array detector (DAD), Thermostatted Column Compartment.
- **Column:** Phenomenex kinetex C18 2.6um, 3.0 x 100 mm

Sample Preparation

Actinomycetes extracts diluted in Ethanol for a concentration of 1mg/mL in a 100 µL insert

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Quick Dereplication Search:

(using mass (MS) data observed and source taxonomic information)

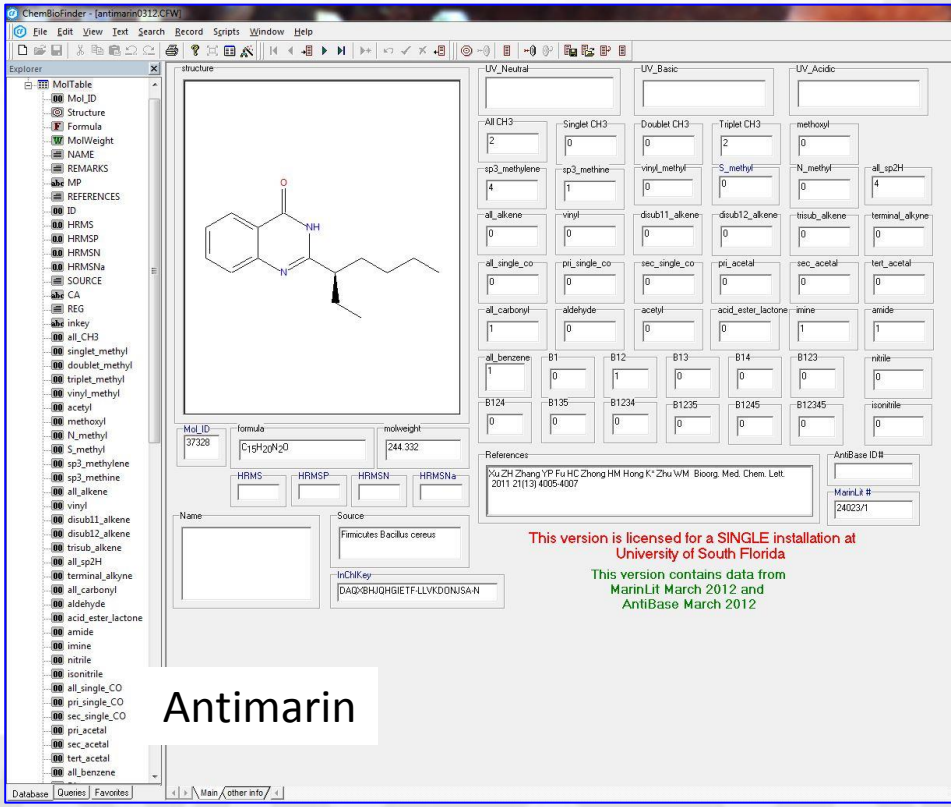
For microbes:

- **Antimarin database (available at the chemodiversity lab)**

  Note (1) the search was based on combined taxonomic and mass information (2) antimarin gathers pure compounds from marine and marine/terrestrial micro-organisms

- **Complementary online data:**

  - Scifinder (exhaustive and fully updated data base)
  - Dictionary of Natural products (not covered by USF)



Antimarin

19-25

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Complex mixture :
m/z+: 381
m/z-: 377

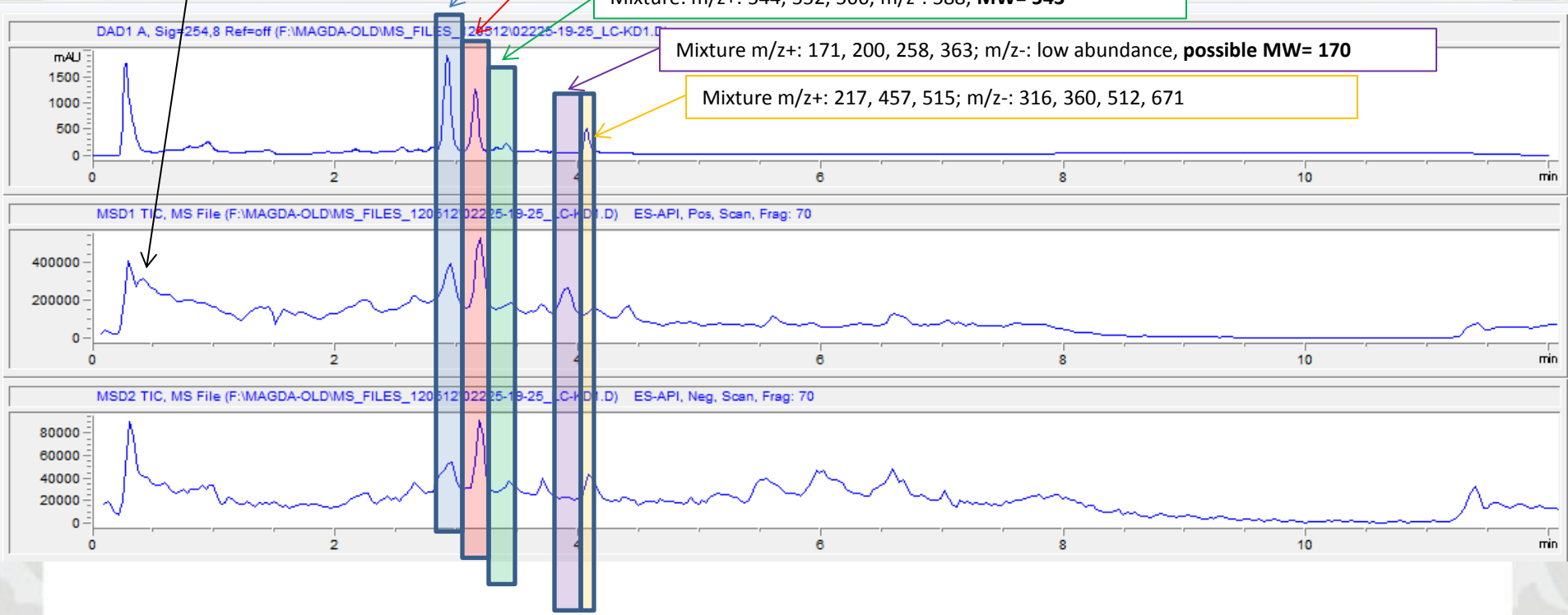
related +/- ions observed: m/z+: 213, 227 (fragments), 498, 516, 538
m/z-: 512 (fragment), 550, 560, **MW= 515**

Pure compound: m/z+: 512, 530, 552; m/z-: 564, 574, **MW= 529**

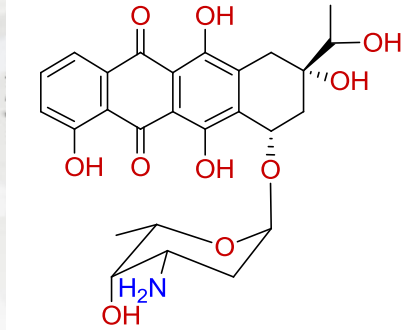
Mixture: m/z+: 544, 552, 566; m/z-: 588, **MW= 543**

Mixture m/z+: 171, 200, 258, 363; m/z-: low abundance, **possible MW= 170**

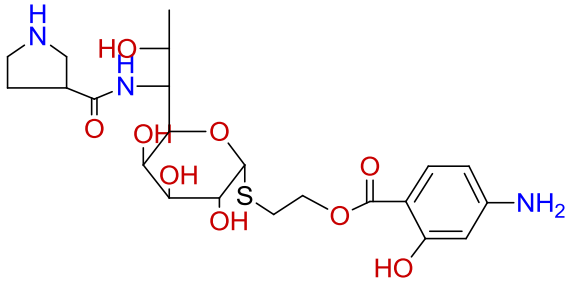
Mixture m/z+: 217, 457, 515; m/z-: 316, 360, 512, 671



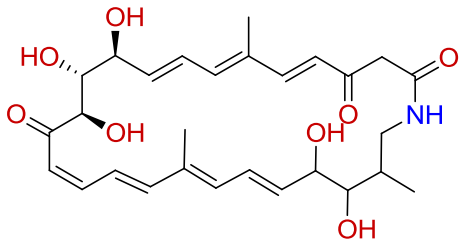
I- possible structures for MW= 515 from sample 19-25 (antimarin)



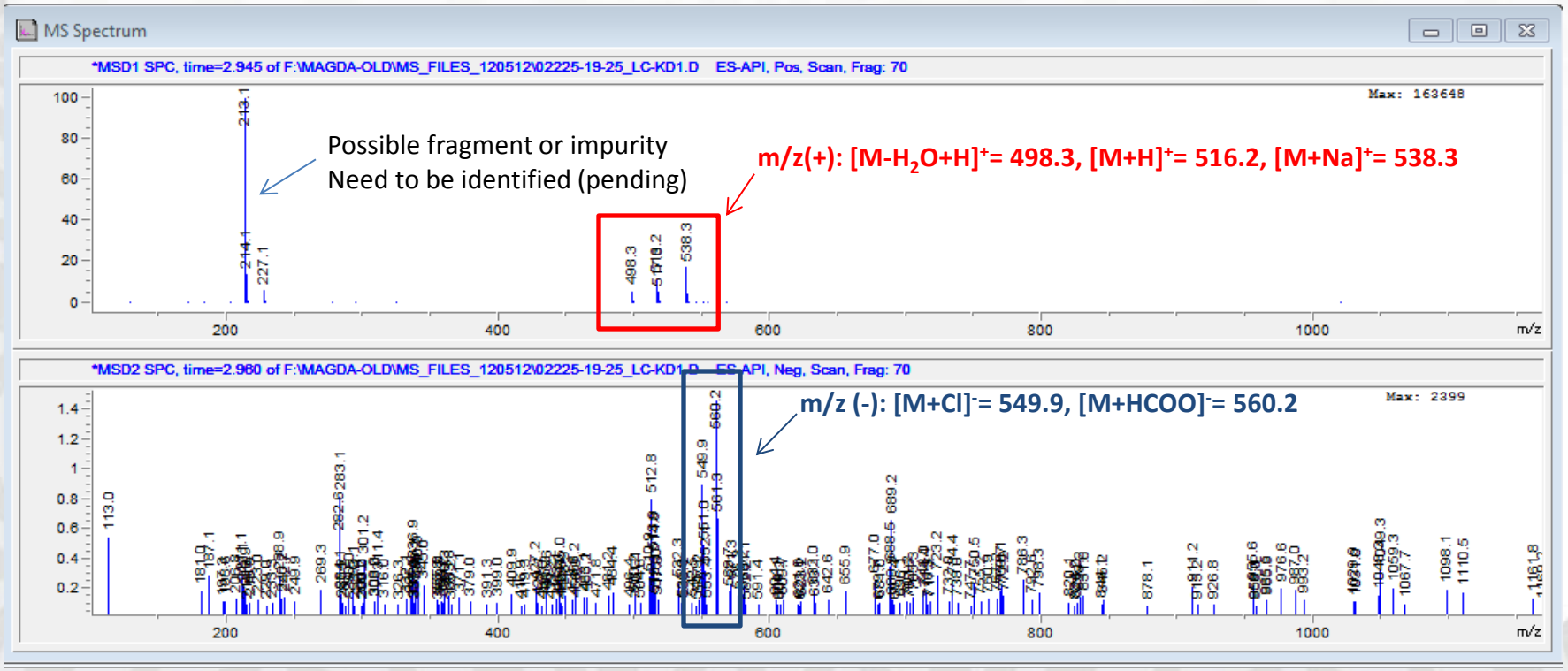
Chemical Formula: $C_{26}H_{29}NO_{10}$
Molecular Weight: 515.5092
10-Hydroxy-13-deoxycarminomycin
(*Actinomadura roseoviolacea*)



Chemical Formula: $C_{22}H_{33}N_3O_9S$
Molecular Weight: 515.5771
N-Demethyl-7-O-demethyldesalicetin
beta-(aminosalicylate)
(*Streptomyces caelestis*)



Chemical Formula: $C_{28}H_{37}NO_8$
Molecular Weight: 515.5953
Sabaramycin B
(*Streptomyces* sp.)



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Summary and Conclusions

- Five extracts from Actinomycetes showed distinct inhibition zones when tested against US MRSA: promising candidates for novel antibiotics
- Initial chemical characterization was performed to investigate their antagonistic properties further
- The established approach and methodologies will be expanded and applied in future studies

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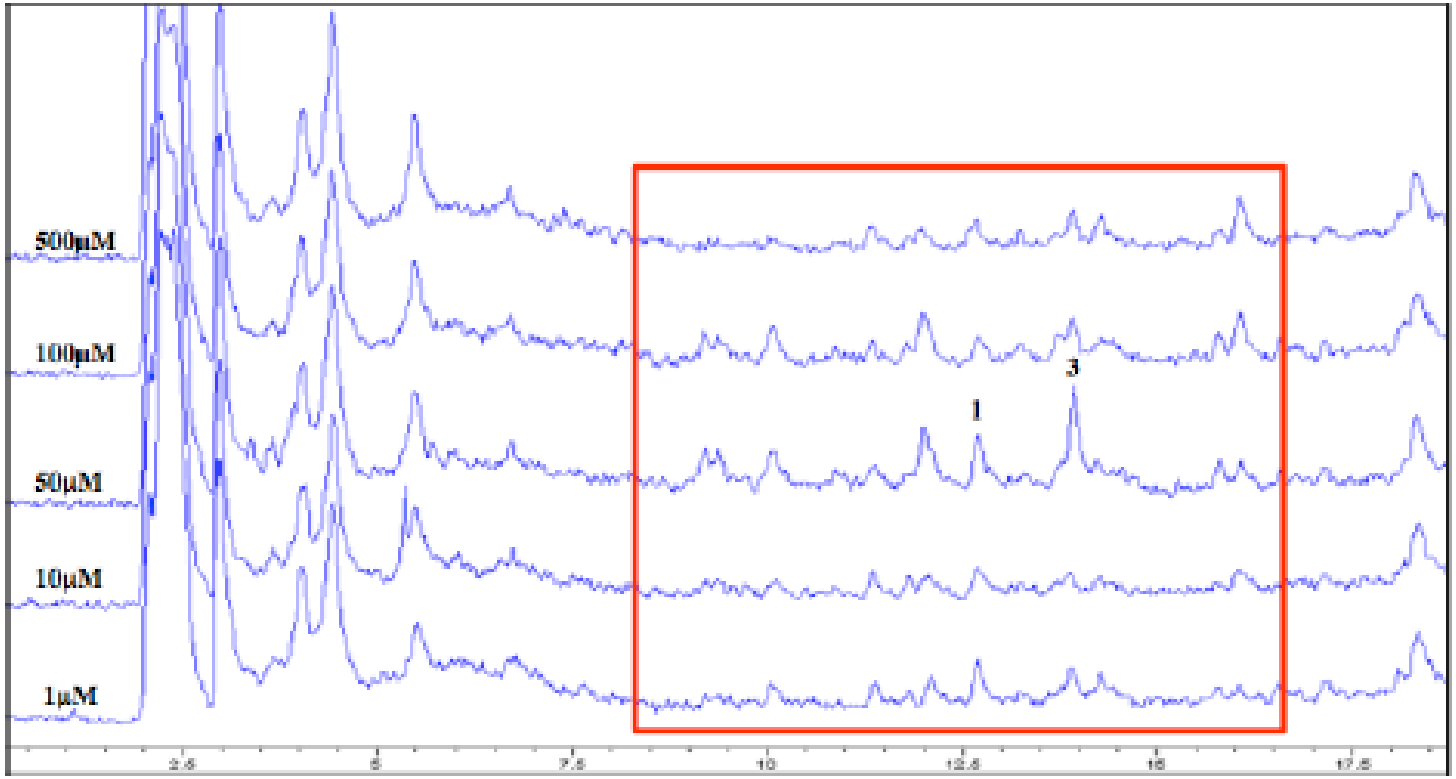
Future directions

- Screen for producers of antibiotics active against *Acinetobacter spp.* and other gram negative pathogens and other important pathogens (MDRTB)
- Approach from several angles---
  - bioactive guided fractionation to test which fractions contain active components
  - chemical characterization to identify novel compound
- Use of PCR and traditional culture to identify producer organism - for scale up
- Identification and gene expression studies of dormant genes
- Differential identification of peaks
  - Prepare large batch culture from different growth conditions
  - Compare extracts from conditions where antibody produced and not producing
  - Identify peaks corresponding to active components

Epigenetic Tailoring for the Production of Anti-Infective Cytosporones from the Marine Fungus *Leucostoma persoonii*

Jeremy Beau [1], Nida Mahid [1], Whittney N. Burda [2], Lacey Harrington [2], Lindsey N. Shaw [2], Tina Mutka [3], Dennis E. Kyle [3], Betty Barisic [3], Alberto van Olphen [3] and Bill J. Baker [1,*]

Figure 3. Overlay of LC/MS total ion chromatograms (TIC) from each concentration of crude extracts of DNA methyltransferase (DNMT)-inhibited cultures. Region highlighted indicates varying levels of the peaks of interest.



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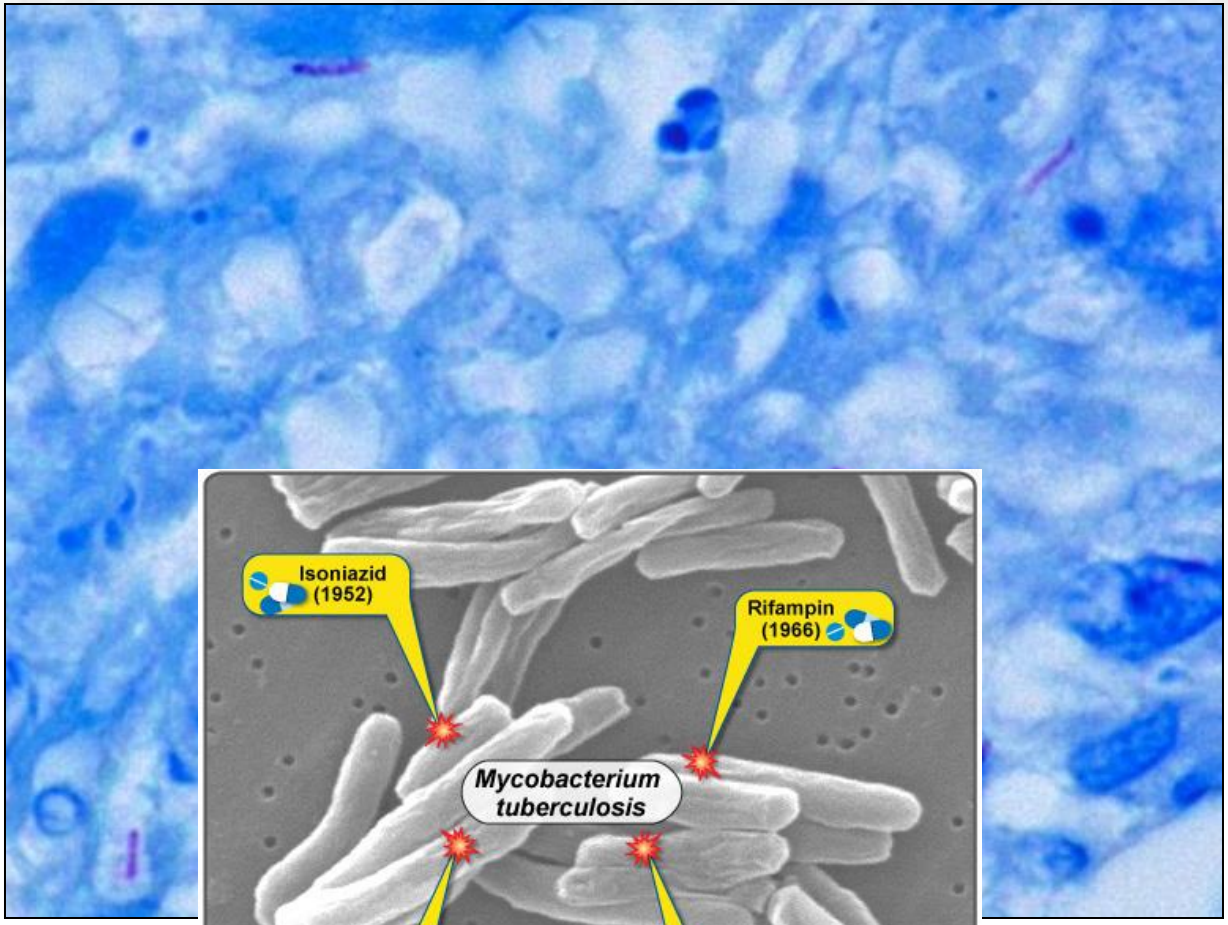
Acid Fast Stain-MDRTB



Acknowledgements

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IMV Collaborator

- Dr. Lyudmila Trezhnovikova
- Faculty & Staff at IMV
- ISTC for funding

Former USF Students

- Lylah Seaton
- Ami Patel
- Colton Faza
- Magda Baksh
- Stefanie Albert

Florida Hospital Collaborators

Jill Whitaker

Christen Mayer

Microbiology Lab

USF Colleagues/CDDI

- Dr. Boo Kwa
- Dr. Jill Roberts
- Dr. Laurent Calcul

The USF College of Public Health for the ECIA and Travel Awards

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Workshop on Biodiversity and Climate Change

Спасибо..!!

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