Microbial Resources: Vital Infrastructure for Research and Extension Endeavors

Maria Auxilia T. Siringan
auxie.nsri@gmail.com

Microbiological Research and Services Laboratory
Natural Sciences Research Institute
University of the Philippines, Diliman, Quezon City

Microbiological Research
and Services Laboratory
Natural Sciences Research Institute
University of the Philippines-Diliman,
Quezon City, Philippines

Vision

A nationally and internationally recognized vital microbial resource center

Mission

Providing quality technical expertise, services and microbial resources to a wide range of clients

Outline

Microorganisms: Resources for Research
Metabolites for the industry

Microorganisms:
Resources for Extension/Technical Services
As tools for assays and efficacy testing

Microorganisms: Resources for Research

Metabolites for the industry

Research endeavors

Microbial diversity studies

- **Exploring metabolic diversity for industrial applications**
- **Screening for useful metabolites**

  As sources of:

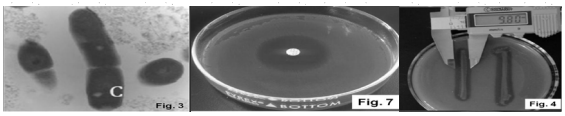
  Enzymes

  Biosurfactant

  Bioactive compounds

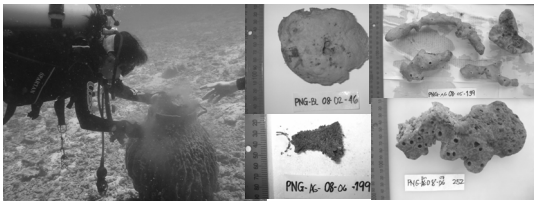
Microorganisms as sources of enzymes

- **Halophilic bacterium from hyperalkaline spring: *Exiguobacterium* sp.**
  Exhibited amylase, pectinase and protease



Microorganisms as sources of biosurfactant

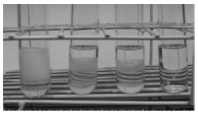
- **Isolates were derived from marine sponges from different regions in the Philippines**



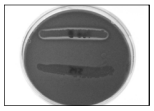
Microorganisms as sources of biosurfactant

- **Screening for biosurfactant production**

Drop Collapse Test



Oil Displacement Test



Hemolysis Test

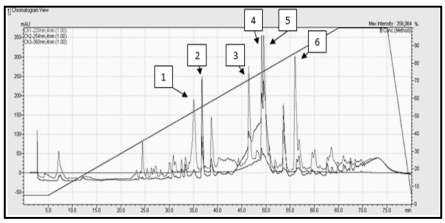
Microorganisms as sources of biosurfactant

- **Extracts of 6 microbial isolates**
  - **Determination of TLC profile**
  - **TLC bands – Test for emulsification activity**
- ***P. aeruginosa* R2A 766B**
  - **Best hemolytic activity**
  - **Determination of HPLC profile**

Sponge-derived MMOs with Biosurfactant Activity	
Identification	**Isolate Code**
Bacillus subtilis	**R2A 515A**
***Gordonia* sp.**	**EM 767A**
Pseudomonas aeruginosa	**ISP3 768A**
Pseudomonas aeruginosa	**R2A-766B**
Pseudomonas stutzeri	**R2A 709A**
Pseudomonas stutzeri	**R2A 685A**

Microorganisms as sources of biosurfactant

- ***P. aeruginosa* R2A 766B**
  - **HPLC Profile of extract – 6 major peaks**

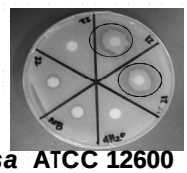


Bradford assay of R2A 766B biosurfactant: possibly a glycopeptide.

Microorganisms from Marine Sponges
As sources of bioactive compounds

Exploring anti-bacterial properties

- **Preliminary screening**
  Disk diffusion assay



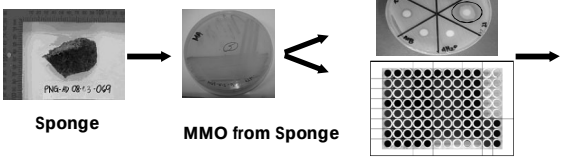
- **Test organisms:**
  ***Pseudomonas aeruginosa* ATCC 12600**
  ***Staphylococcus aureus* ATCC 10145**

Microorganisms from Marine Sponges
As sources of bioactive compounds

Exploring anti-bacterial properties

Isolation of Microorganism

Antimicrobial Screening

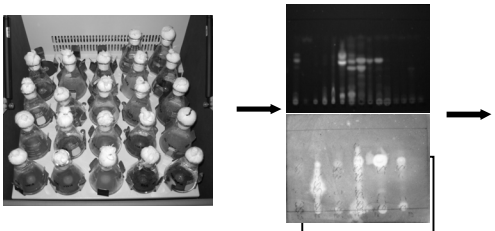


Sponge

MMO from Sponge

Microorganisms from Marine Sponges
As sources of bioactive compounds

Exploring anti-bacterial properties

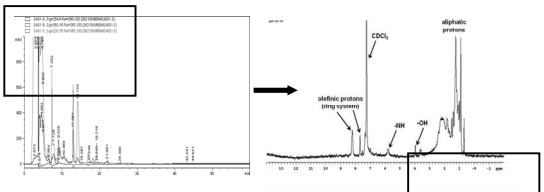


Initial Anti-infective Production

TLC Assay of extracts of Bioassay of TLC Fractions

Microorganisms from Marine Sponges
As sources of bioactive compounds

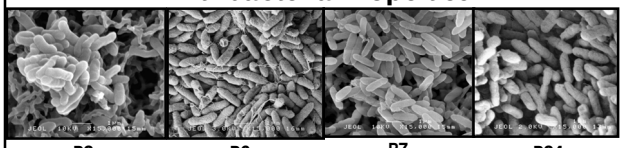
Exploring anti-bacterial properties



HPLC Analysis

Structure Determination: MS/NMR

Microorganisms from Sponges: Anti-bacterial Properties

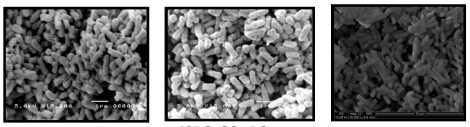


R3 *Chryseobacterium indologenes*

R6 *Bacillus subtilis*

R7 *Stenotrophomonas maltophilia*

R24 vs. *S. aureus*



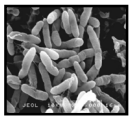
ISP2-1-A

ISP2-23-A2

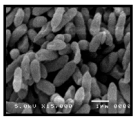
SDA-15-A

Identification of all isolates: *Pseudomonas aeruginosa*

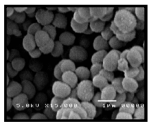
Microorganisms from Sponges: Anti-bacterial Properties



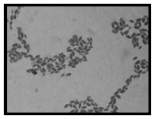
P142
Achromobacter xylosoxidans



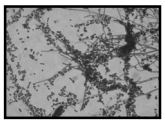
SDA-430-A
Pseudomonas oryzihabitans



ISP2-602-A
Micrococcus luteus



R2A-512-
Micrococcus luteus



ISP2-569-A
Streptomyces sp.

Antimicrobial Production by ISP2-569-A

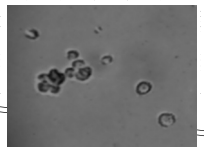
***Streptomyces* sp.** (add flasks in NSRI video)

Optimization studies: Determination of C and N sources for enhanced yield of antimicrobial compound from ISP2-569-A

Microorganisms from Marine Sponges As sources of bioactive compounds

Exploring anti-protozoan properties

- **Determination of % reduction in the viable *Trichomonas vaginalis* cell count**



Trichomonas vaginalis

Microorganisms from Sponges: Potential anti-protozoan compounds vs. *Trichomonas vaginalis*

Sponge-derived MMOs	Isolate Code	Methanolic Extracts: Percent Kill of *T. vaginalis* (%)	Acetone Extracts: Percent Kill of *T. vaginalis* (%)
Achromobacterxyloxosidans	P-142A-I-1-A	96.7	99.5
Chryseobacterium indologenes	P-046-I-1-A	97.2	95.7
Micrococcus luteus	ISP2-602-A	98.1	99.1
Penicillium griseofulvum	ISP2-576-A	99.3	99.8
Pseudomonas aeruginosa	ISP2-1-A	100.0	98.0
Pseudomonas aeruginosa	ISP2-23-A2	92.6	99.4
Pseudomonas oryzihabitans	SDA-430-A	97.8	99.2
Rhodoturula mucilaginosa	R2A-512-B	99.6	97.2
Stenotrophomonas maltophila	P-203-I-1-A	98.0	99.1
Streptomyces rubiginosohelvolus	ISP2-569-A	99.8	99.9

Microorganisms: Resources for Extension and Technical Services

As tools for assays and efficacy testing

Extension and Technical Services

Test organisms – Tools for Efficacy Testing

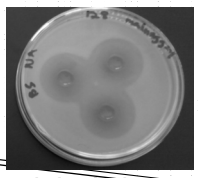
- For the evaluation of materials and products with biocidal agents
- For the evaluation of sterilization technology

- Test organisms – most are ATCC cultures

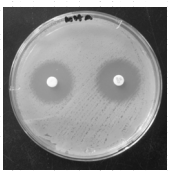
Test Organisms	Accession Numbers
Bacteria	
Bacillus subtilis	ATCC 6633
Escherichia oli	ATCC 25922
Pseudomonas aeruginosa	ATCC 15442; ATCC 27853
Salmonella typhimurium	ATCC 14028
Staphylococcus aureus	ATCC 6638; ATCC 29213
Fungi	
Aspergillus niger	
Candida albicans	ATCC 10231
Trichophyton mentagrophytes	UPCC 9533

- Test organisms: Applications

Antimicrobial Assay
Qualitative screening of natural and synthetic compounds

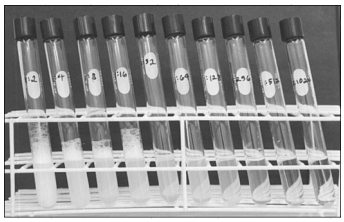


Antimicrobial assay of *Moringa* extract



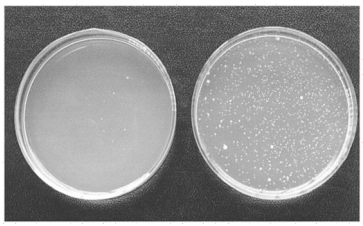
Antimicrobial assay of *an antibiotic formulation*

- Test organisms: Applications



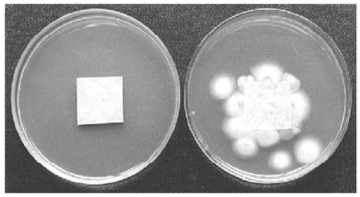
Determination of Minimum Inhibitory Concentration (MIC) of Biocidal Agents

- Test organisms: Applications



Percent kill Test
Efficacy testing of Disinfectants and other biocidal-containing materials and formulations

- Test organisms: Applications



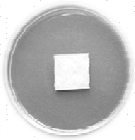
Mold Resistance Test
American Society for Testing and Materials
ASTM Designation: D-4576-01

Determination of efficacy of antifungal agent in the material/matrix

- Test organisms: Applications

Materials tested include:

Baseball glove

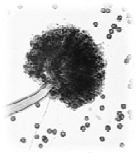


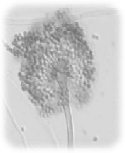
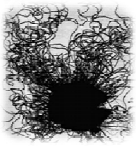
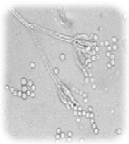
Packaging material
(e.g. soap box)

Military boots

Mold Resistance Test
Fungal species used as Test organisms

Aspergillus niger ***A. terreus*** ***A. ustus***



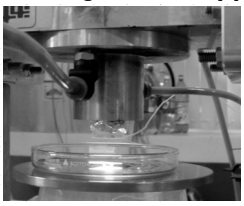
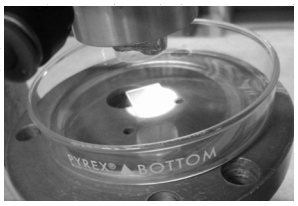




Chaetomium globosum ***Penicillium frequentans***

- **Test organisms: Applications**

Preservative Challenge Test
Determination of efficacy of preservation of various formulations and packaging materials

- **Test organisms: Applications**

Plasma Technology
As alternative sterilization procedure for food, packaging materials, & medical devices
Efficacy testing of plasma technology as sterilization procedure

Challenges and future directions

Sustainability of collection of microbial Cultures
Problem: Loss of cultures
Loss of activity in viable culture
Harnessing the potential of microorganisms
Problem: Sustainability of endeavor

Major needs: Funds, facilities and equipment, manpower

Future directions

- **Upgrading microbial holdings**
- **Sustainable long-term preservation**
- **Upgrading of facilities to support culture collection**
- **Enhancing database**
  - **Data generation:**
    - **Phenotypic traits**
    - **Genotypic traits**
    - **Useful genes through genome sequencing**
- **Capacity building**
  - **Training in key techniques used in microbial systematics**
  - **Training in metagenomics relevant to metabolite production**