HEALTH AND BIOTECHNOLOGY

The Role of Microbial Genomics

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Lecture Outline

- Overview
- The Convergences of the “-omes”
- Health Applications of Microbial Genomics
- Our Experience and the Importance of Biobanking

Overview

- What is Health?
  - Absence of infirmity
    - Physically and Mentally
  - Is both a local and international matter, one shared across the world, among many nations and populations
  - Our own health concerns impact our neighbors and vise-versa

Overview

- Biotechnology
  - is the use of biology or biological processes to develop helpful products and services.
  - is the set of biological techniques originally resulting from basic research, specifically molecular biology and genetic engineering, and now used for research and product development.
  - Alternatively, biotechnology can be defined as the scientific manipulation of organisms at the molecular genetic level to make beneficial products (Microbial Biotechnology).

Overview

- Microbes
  - are the oldest form of life on Earth.
  - Although they are considered simple organisms and are too small to be seen with the naked eye, microbes are extremely diverse and adaptable.
  - Their impact can be negative, such as causing disease, or positive such as maintaining the Earth's atmosphere and promoting plant growth.
  - They are the source of many products, including enzymes for research, antimicrobial agents, and antibiotics

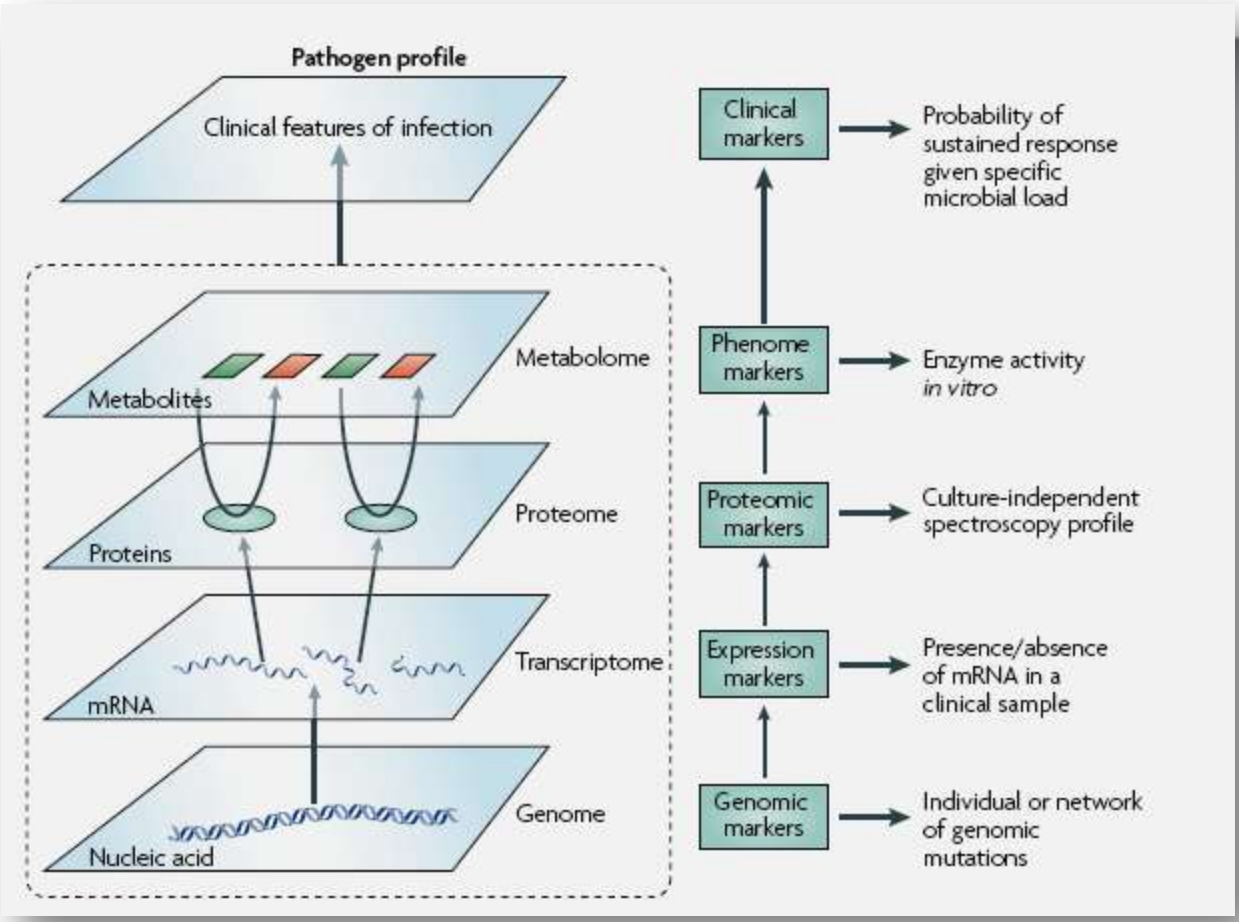
Overview

- Microbial Genomics
  - The study of microbial genomes (all the genetic information in the microbe) enables scientists both to understand how microbes live and to isolate microbial genes for use in biotechnology.
  - The combination of microbial genomics and biotechnology is leading to development of new diagnostic tools, better vaccines, improved treatments for disease, better detection of pollutants, and cleanup of contaminated environments.

The Convergence of the “-omes”

- The completion of the Human Genome Project heralds the dawn of the new era
  - The Post-Genomic Era

The Convergence of the “-omes”



Profiling the enemy by “bottom-up” Approach…

HEALTH APPLICATIONS OF MICROBIAL GENOMICS

Microbial Genomics for New Antibiotics

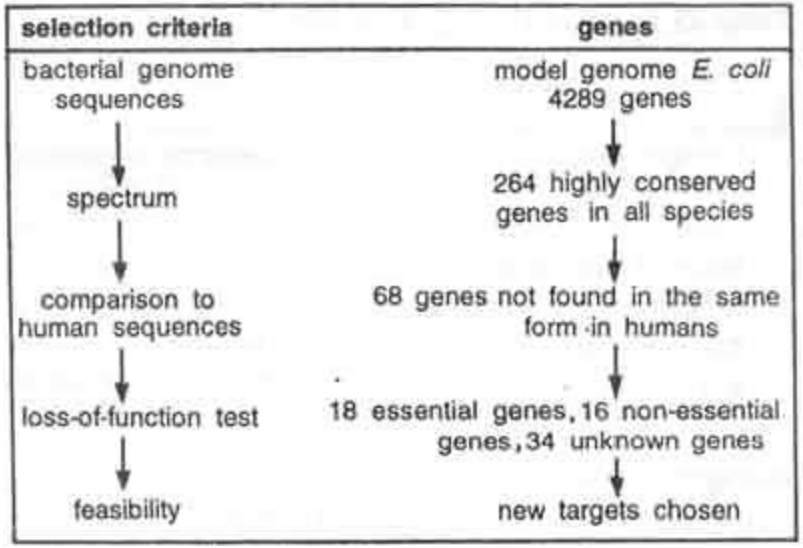
- Development of antibiotics, in recent past has witnessed a shift from direct antimicrobial screening towards rational target based strategies.
- This shift is the result of availability of, massive data from genomics research involving whole-genome sequences study of expression profiles and proteomics .

Microbial Genomics for New Antibiotics

- Microbial genomics has already facilitated the ability of the pharmaceutical industry in hunting for antimicrobial drugs particularly when the old drugs become ineffective due to following reasons:
  - development of resistance in microbes against the antibiotic, sometimes involving multiple systems, which reduces the effect of an antibiotic
  - a new range of organisms becoming potential pathogens due to longevity of patients

Microbial Genomics for New Antibiotics

- Target Based Screening



Steps involved in target identification for a respiratory tract antibacterial Drug

Microbial Genomics for New Antibiotics

- Target Based Screening
  - Advantages:
    - 1. More sensitive;
    - 2. Easy screening;
    - 3. Rational drug design;
    - 4. Low toxicity
  - Disadvantages:
    - 1. In vitro inhibitor needs to be converted into an antibacterial drug (problem of penetration);
    - 2. Genetic validation (by gene knockout) can be misleading.

Microbial Genomics for Diagnostics

- Platforms evolution
  - Microscopy and Culture
  - Antigen and Antibody Assay Detection
  - Nucleic Acid Testing
  - Multiplexing
  - The future is now!
    - Syndromic Diagnostic Panel
      - Viral Meningitis Panel
      - Mosquito Panel
      - FUO Panel
      - Respiratory Panel
    - Resistance and Genotypic testing

Microbial Genomics for Treatment

- Oral Bacteriotherapy
- Vaccines
- Monoclonal Antibodies

Our Microbial Genomics Program

- Flagship Projects
  - Dengue
  - TB
  - Influenza
  - Human Microbiome & Malnutrition
  - Metagenomics and Climate Change
  - Biosurveillance Program for Emerging Infectious Diseases
  - MDRO Surveillance

Dengue Genomic Program

- 3 Arms
  - Diagnostic Applications
    - Isothermal Technology
    - Nucleic Acid Lateral Flow Assay
  - Phylodynamic Studies

FIELD-BASED RAPID DIAGNOSTIC KIT FOR THE EARLY DIAGNOSIS OF DENGUE INFECTION

In-House LAMP Diagnostics for Dengue Virus Infections in the Philippines (The Biotek-M Project)

Objectives:

- design a loop-mediated amplification (LAMP) diagnostic protocol that will be employed as a routine tool for simultaneous detection and serotyping of dengue virus infection in portable miniaturized platform.
- design the serotype-specific oligonucleotide primer sequences that can be used in the LAMP protocol
- perform Comparative Performance testing using Commercial LAMP assay vs. In-house LAMP assay using sequencing as the gold standard

Expected Output

- This project is expected to produce a low-cost and simplified diagnostic kit for the rapid detection and simultaneous serotyping of dengue virus infection.

Results

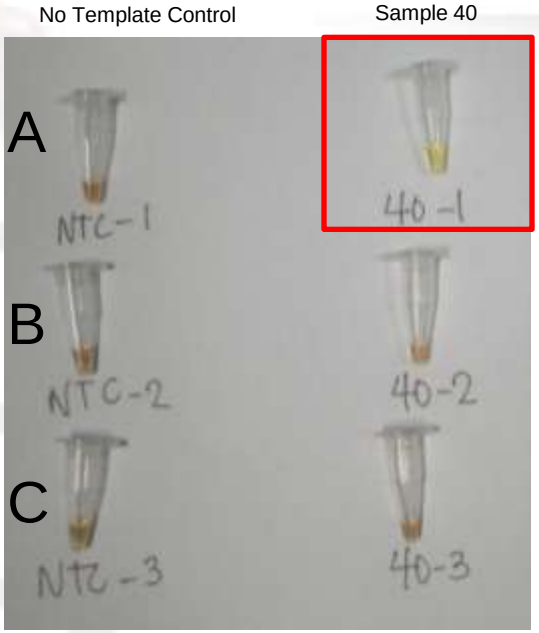


Figure 1 shows the LAMP assay of Sample 40 which is Serotype 1. (A) Using DENV 1 primers. (B) Using DENV 2 primers. (C) Using DENV 3 primers.

(A) shows a change in color which means that primers for DENV1 are working.

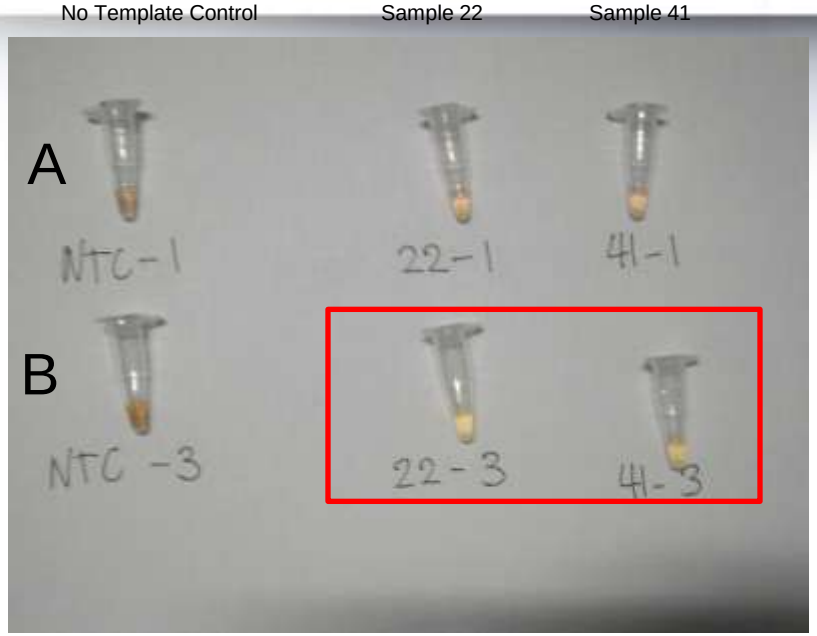


Figure 2 shows the LAMP assay of Samples 22 and 41 which iare both Serotype 3. (A) Using DENV 1 primers. (B) Using DENv 3 primers.

(B) shows a change in color which means that primers for DENV3 are working.

Both assays have the same reaction conditions which means that both can be done simultaneously in one LAMP Heater. The primers for DEN 2 and DENV 4 are being re-designed due to problems in cross-reactions.

Dengue Genomic Program

- Phylodynamic Studies, Climate Change and Mathematical Modelling
  - The melding of immunodynamics, epidemiology and evolutionary biology to explore how pathogen genetic variation is modulated by host immunity, transmission bottlenecks and epidemic dynamics to determine the wide range of pathogen phylogenies observed at scales from individual host to population.

A New Potential Dengue Genotype from Serotype 3 strain

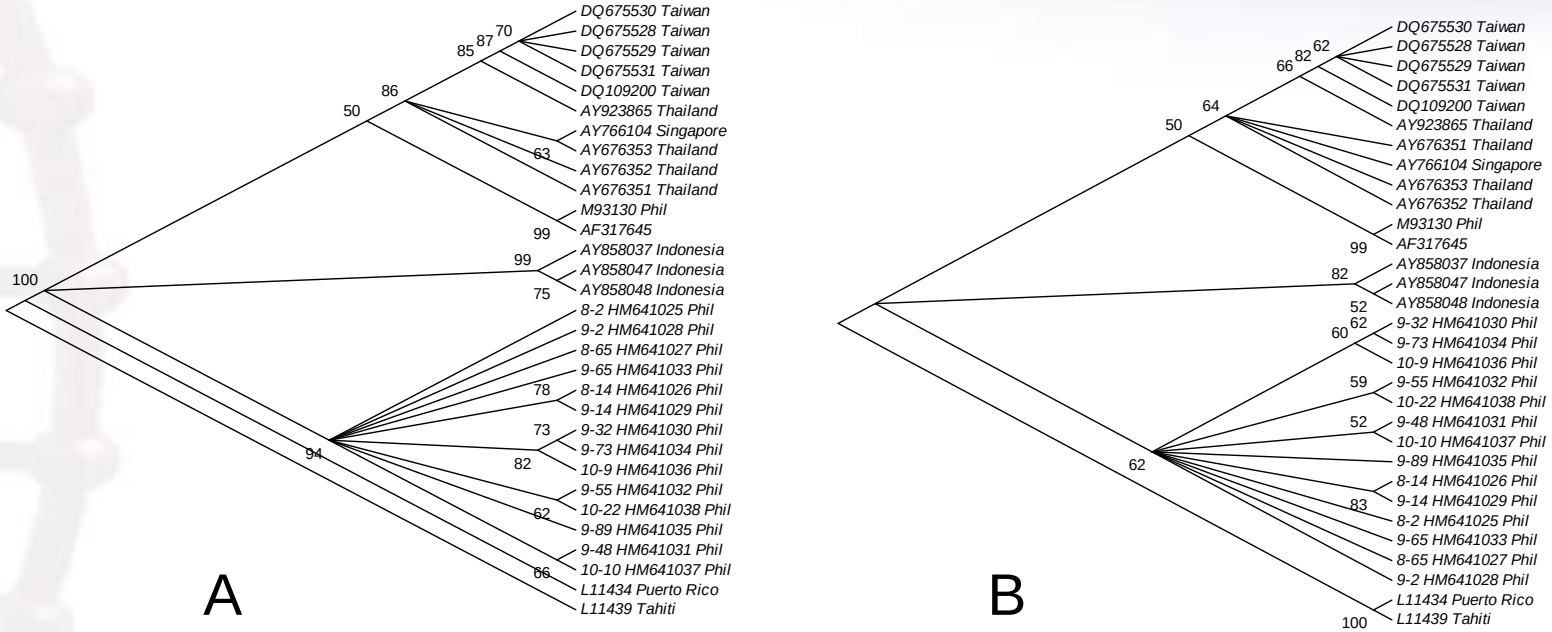


***Figure1*. Phylogenetic analysis of the CprM genes from 31 DENV-3 isolates using Neighbor-Joining Method (A) and Maximum Parsimony (B)**

Development of Research Matrix

Dengue Virus	Genomics	Mixing	Mutations	Virulence
Dengue Vector	Migration	Mixing	Rapid Propagations	Effective transmitter
Climatic Conditions	Temperature	Rainfall	Storm Events	Inter-annual climate variability
Rural-Urban Development	Changing habitat	Efficient reservoir	Density of resting sites	Higher population = more number of cases
Disease Outbreaks	Changing pattern of case density	Multiple serotypic exposures	Public health breakdown	Increasing mortality trends

TB GENOMICS PROGRAM

- MDR AND XDR DiagnosticS
- Resistance Induction and Gene Expression

Biotek-M Program

Field-Based Rapid Diagnostic Kit for the Early Detection of Dengue Infection

Biotech-Manila Molecular Diagnostics Program for the Early Diagnosis of Tuberculosis

The study will include…

I. Whole Genome Sequencing

II. In-vitro models of induction of drug-resistance of *M. tuberculosis*

III. Diagnostic Technology for the Detection of MDR- and XDR-TB

IV. Clinical Validation

MICROBIAL GENOMICS AND OUTBREAK INVESTIGATION

MOLECULAR FINGERPRINTING OF MDR-PSEUDOMONAS AERUGINOSA CLINICAL ISOLATES FROM A TERTIARY HOSPITAL FACILITY

The Story

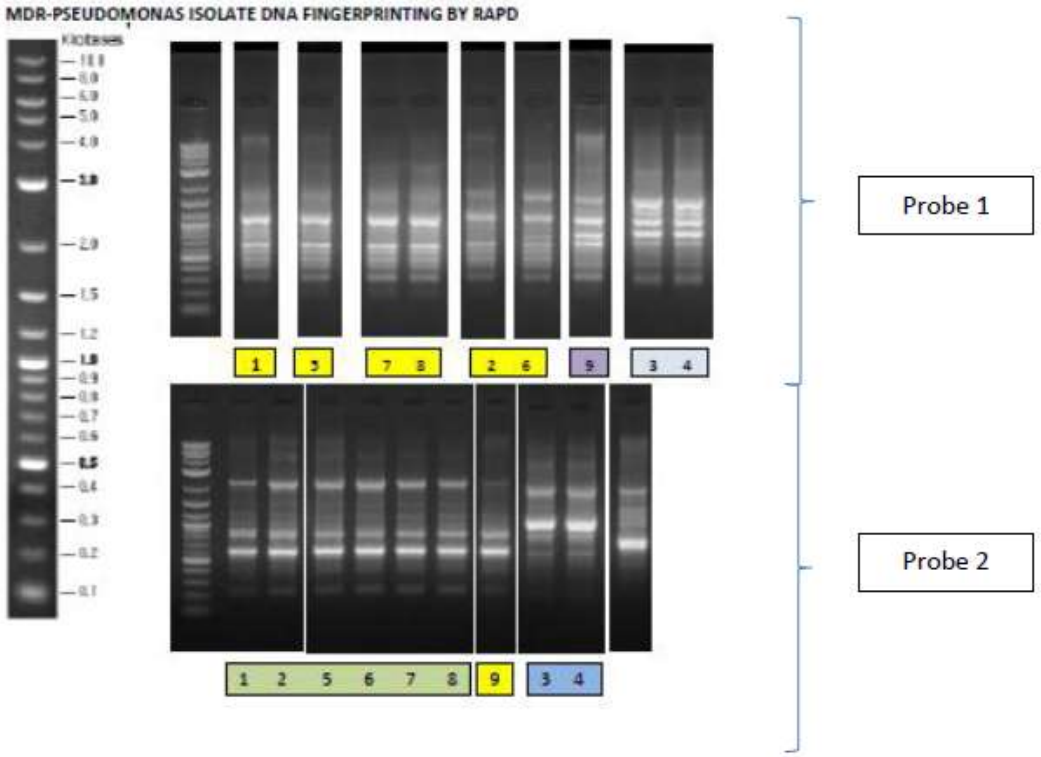
- Early June of 2010, a few cases of MDR-Pseudomonas aeruginosa (MDR-PDA) were isolated from clinical specimens
- Initial investigation revealed cases clustering in one critical care unit
- Environment sampling and rectal swabbing of patients co-admitted with the positive cases were done to document colonization –NO SUCH ISOLATE WAS IDENTIFIED
- Review of all MDR-Isolates was done by HICC and Clinical Microbiology showed 13 cases identified. 12 were from tracheal aspirates and one from a urine sample.
- Isolates stored from the MDR and XDR biobank facility were reviewed and 9 of the 13 clinical isolates from the identified cases were successfully revived
- Repeat Phenotypic resistance testing were done to confirm the resistance pattern and all revealed resistance to all except colistin using the Vitek 2 system
- All 8 clinically relevant isolates were subjected to fingerprinting

Results

- 3 genotypically distinct clones were identified from the 9 isolates using Random Amplification of Polymorphic DNA method (RAPD)
- Clones were labeled based on the order of appearance
  - o C1
  - o C2
  - o C3

Results

PHOTO 1: Electrophoretic band separation patterns of the 9 isolates plus one drug-susceptible control. Images were re-arranged according to banding pattern similarity using to distinct molecular probes. A difference of 3 banding patterns were considered significant. TMC 1 Clones were from samples 1, 2,5,6,7,8 ; TMC2 clones were from samples 3 & 4 (came from one patient source); TMC3 clone is from Sample 9.



Results

- 6 out of 8 isolates were all from the same clone suggesting the occurrence in-hospital transmission of the organism starting from patient no. 1 who was admitted in January of 2010.
- Samples two and three have very distinctive pattern which was found to have a similar banding pattern from a representative isolate from another hospital

Implications

- There was a break in infection control practices
- Hospital transfers does not only entail transfer of patients but also transfer of organisms they carry with them

Microbial Genomics and Biobanking

- Recent advances in molecular high-throughput assays have intensified the need for well annotated, properly preserved biospecimens.
- A thorough assay often requires samples from both diseased/treated and normal/untreated tissue.
- The Science of BioBanking addresses methodologies that maximize the quality and utility of biospecimens for biomarker research.

Who should perform biobanking?

- Research scientists working with:
  - Biospecimens
  - Biomarkers
  - Bioextraction
  - Biopreservation

At our institute…

- Biobanking bar coding system for:
  - DNA and RNA extracts
    - Viruses (HBV, HCV, DENGUE, INFLUENZA)
    - Salmonella, Entero-aggregative E. coli
    - C. difficile
    - Enteroprotozoans (Cryptosporidium, Giardia and Entamoeba)
    - TB
  - Plasma, Serum and whole blood samples
  - Stool

Conclusions

It has been argued that:

- There is no substitute for a detailed understanding of microbial physiology
- Target selection must include a determination of what level of inhibition is required to attain clinically effective growth inhibition and/or bactericidal activity
- Bacterial genomics can identify novel targets and help develop better assays.

Conclusions

Genomics may be viewed as an enabling tool to:

- Identify pathogens responsible for acute and chronic disease;
- Select antimicrobial targets;
- Understand drug synergies and antagonism;
- Improve pharmacokinetics;
- Target the niche of a pathogen and its genomic responses to ecological cues and stress
- Understand and avoid resistance

Challenges

- The methods used to generate input data and

  standards for sharing data are still evolving.

- A shift of emphasis towards integrative data analysis and sharing is difficult, but might prove to be the key to the successful translation and integration of laboratory diagnostics medical therapeutics into improving clinical and public health outcomes in medicine.

Thank you!

- Questions??