Bioterrorism, Biocrime and Microbial Forensics

BARRY SAVILLE, PHD

ASSOCIATE PROFESSOR FORENSIC SCIENCE PROGRAM

Course Evaluation Online

- I hope you will take the opportunity to provide feedback.

Novel Coronavirus (SARS-CoV-2) – Coronavirus disease (COVID-19)

Although it is interesting to discuss and think about the current Pandemic in the context of this lecture we really do not know enough to determine how or when we will be on the other side of this and so I will leave the discussion to those with more experience. If you are interested, this article by Time magazine based on an interview with Dr. Bruce Aylward, a Canadian physician-epidemiologist who is senior adviser to the Director-General of the World Health Organization (WHO), is quite good

https://time.com/5805368/will-coronavirus-go-away-world-health-organization/

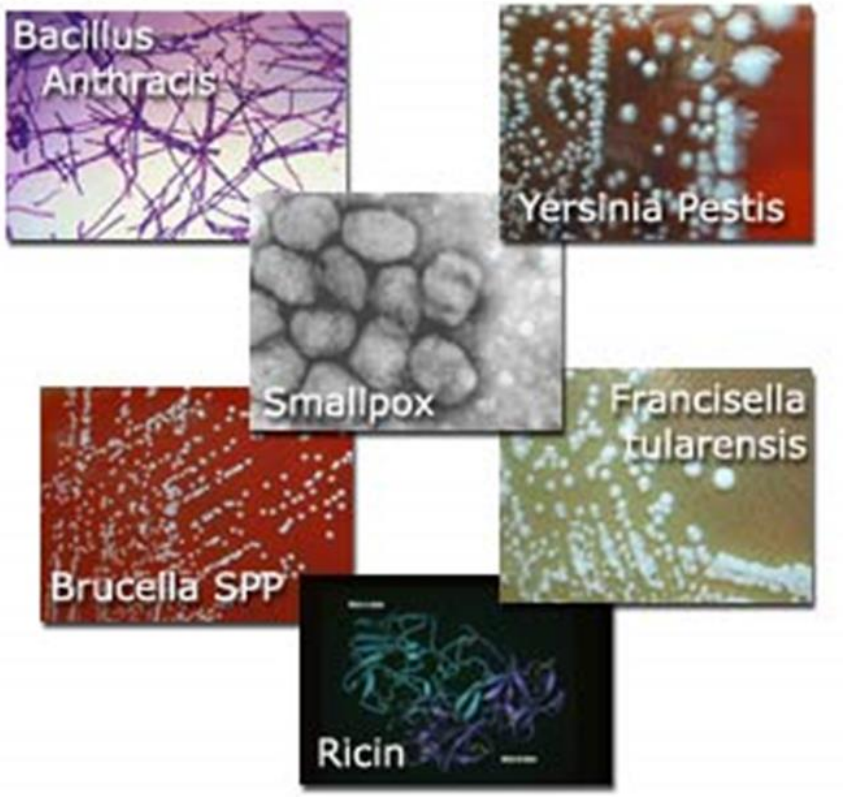
Lecture Outline

The biological agents, and the diseases

Terrorism

Why biological agents

Microbial Forensics



Biological (bioterrorist) Agents

Viruses

Bacteria

Fungi

Biological Toxins

Viruses

Viruses are molecular parasites grouped by genome organization

- no metabolism of their own
- They infect all biota of the planet

Filterable agents

- 2- 60-fold smaller than the gram-positive bacterium *Staphylococcus*

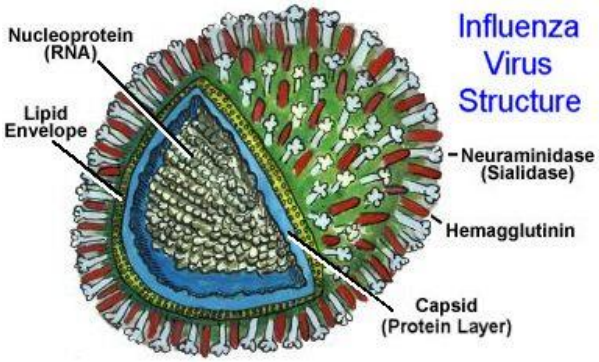
Major Components of a Conventional Virus

Nucleic acid genome

- codes all virus specific macromolecules

Capsid

- Protein shell, protects and delivers the genome



http://www.scientificpsychic.com/health/virus.html

Envelope (some viruses)

- lipid envelop derived from the host cell,
- membranes contain virus derived glycoproteins that have a role in viral tropism (the host organ specificity of a virus)

21 families infect humans

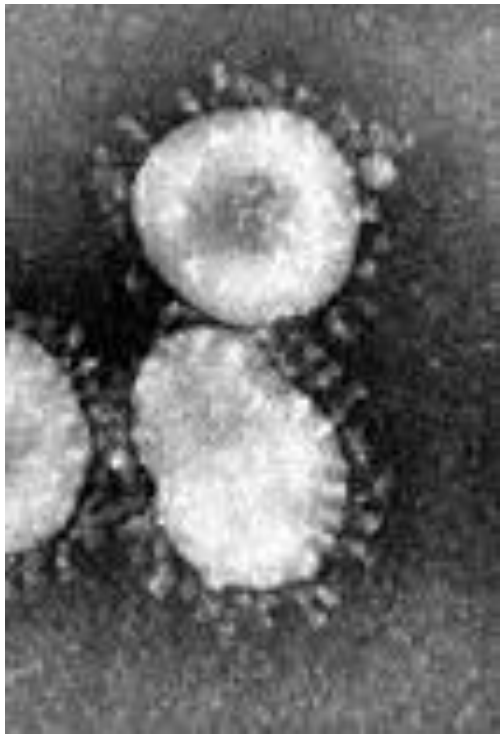
Respiratory viruses

- Rhinovirus - common cold, member of the *Picornaviridae*, single-stranded positive sense RNA virus
- There are at least 160 different strains, or serotypes, of rhinovirus
- Influenza member of the *Orthomyxoviridae*,enveloped virus, segmented, negative-strand RNA genomes
- 1918 flu pandemic (Spanish Flu) 20 million plus died

Image of a Spanish flu ward Fort Riley, Kansas,, https://en.wikipedia.org/wiki/Spanish_flu

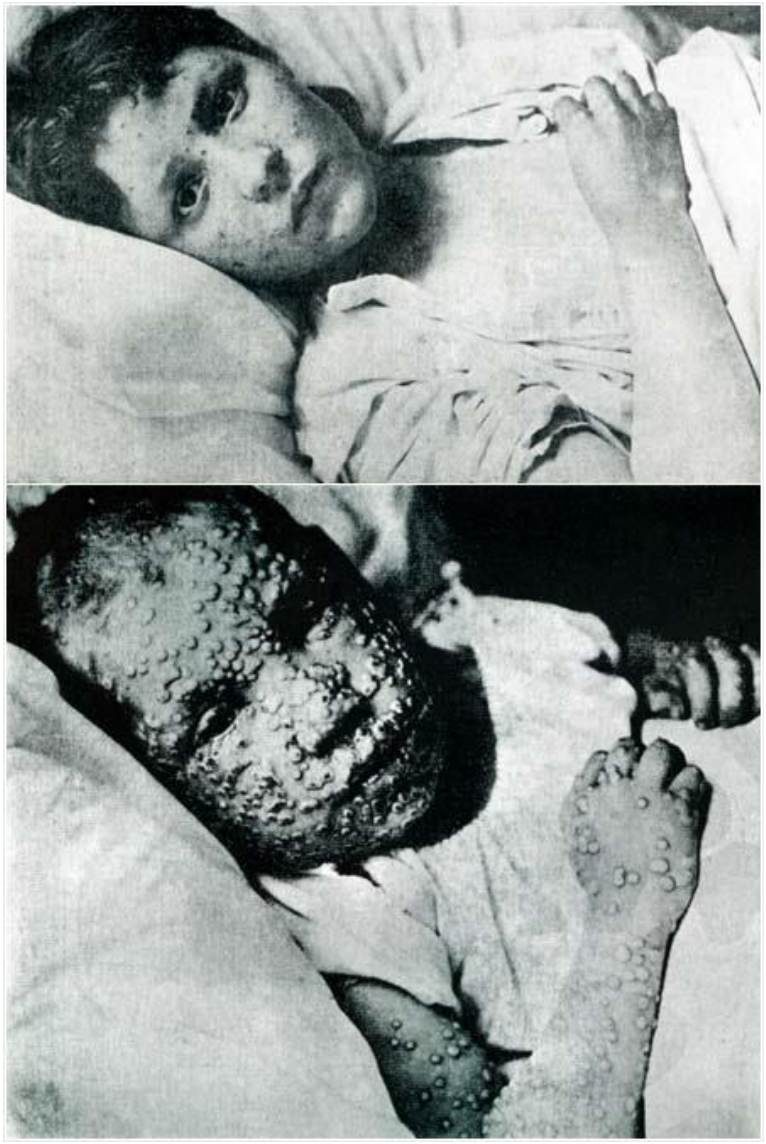
Coronaviruses

- Enveloped viruses
- Positive-sense single-stranded RNA genome
- Nucleocapsid of helical symmetry.
- Genome Size 27 to 34 kilobases, the largest among known RNA viruses.
- “from the Latin corona, meaning "crown" or "halo", which refers to the characteristic appearance reminiscent of a crown or a solar corona around the virions (virus particles) when viewed under two-dimensional transmission electron microscopy.”



https://en.wikipedia.org/wiki/Coronavirus

Hemorrhagic fevers and Small Pox



Ebola Patient (Intensive Care)

http://news.asiantown.net/r/37593/

The pustule covered hand of a baby with a mild case of smallpox. CDC

http://www.teara.govt.nz/en/photograph/30318/smallpox

Smallpox as a Biological Weapon

Variola virus is highly contagious, spreads easily by contact or via aerosol from one human to another

Virulence could be enhanced by genetic engineering, the whole virus can be synthesized

Attack against USA could threaten the whole world resulting in the deaths of tens of millions and paralyzing industrial economies

Bacterial Pathogens

Bacteria are living cells

- Capable of complex metabolic functions
- Larger more complex genomes than viruses
- Cell walls, membranes surrounding a cytoplasm and nucleoid

Bacteria Considered Bioterrorist Agents

Biowarfare agents – aerosolize

- *Bacillus anthraxis* (anthrax)
- *Yersinia pestis* (plague)
- *Francisella tularensis* (tularemia)
- *Burkholderia sp*. (glanders and melioidosis)
- *Brucella sp.* (brucellosis)

http://socyberty.com/issues/anthrax-3/

Agents that are food borne pathogens, *Shigella* and *Salmonella* (biocrime attacks)

- For further information see the following web site and links on it http://www.phac-aspc.gc.ca/id-mi/index-eng.php

Yersinia pestis

Three plague pandemics, one from 541-750 called the Justinianic, the Black Death, which began in the 1340s, and a third started in Hong Kong in 1894 from which point it is thought to have spread around the globe (Green et al 2014) – Madagascar

The black death alone lead to the death of 50 million people in 4 years, 60 percent of Europe's population

Bacterial Toxins

Botulinum neurotoxin – *Clostridium botulinum*, the most potent natural toxin known

Staphylococcus aureus toxins

Tetanus toxin - *Clostridium tetani*

Also: Alpha toxin, Anthrax toxin, Cyanotoxin, Diphtheria toxin, Exotoxin, Pertussis toxin, Shiga toxin, Shiga-like toxin

Fungal Diseases

Many human mycoses

Fungal infections of humans are increasingly becoming a problem for immuno-compromised individuals

The majority of plant diseases are caused by fungi, losses in the billions of dollars

Fungi used to infect plants can have a significant impact of society

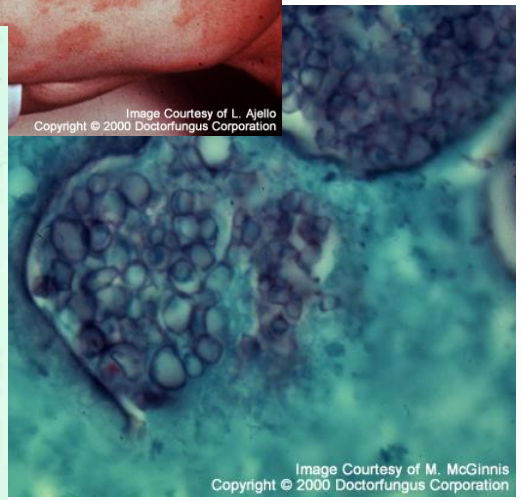
Fungi Impacting Humans

Wheat Stem Rust, *Puccinia graminis* f. sp. *tritici*, USDA CDL

Wheat Leaf Rust, *Puccinia tritici*, USDA Cereal Disease Laboroatory

Cryptococcus neoformans

Ergot of Rye, *Claviceps purpurea*



Coccidioides immitis

Historic, Plant Pathogen Epidemics

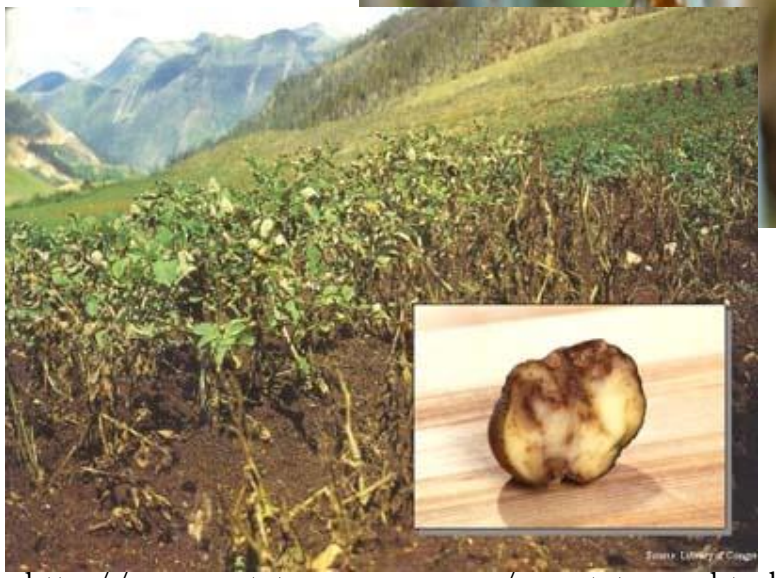
CIMMYT

The 1845-1846 potato blight epidemic in Ireland killed 1 million people and lead to the emigration of 1.5 million (Carefoot and Sprott. 1967.

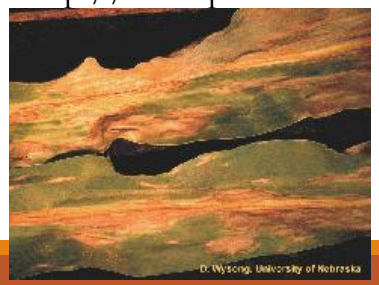
- Documentary "Death or Canada"

1970 Corn leaf blight - 20% U.S. crop loss ~ $1 billion

Currently *Puccinia graminis* f.sp *tritici*, wheat stem rust, new races e.g. Ug99, no cultivated resistance, pathogen of biblical proportions (not in Canada yet)



http://www.potatomuseum.com/expotatoyear.html



http://nu-distance.unl.edu/homer/disease/agron/corn/CoSCLB.html

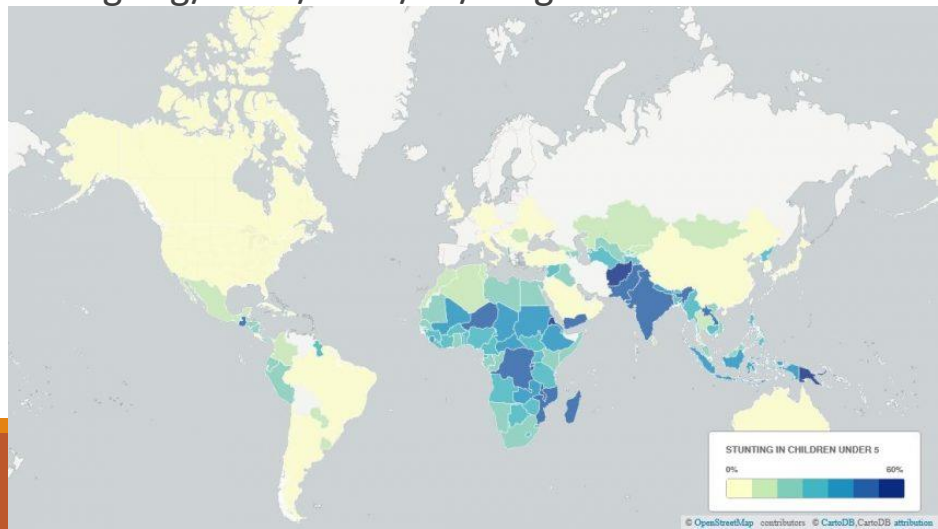
Fungal Toxins

Fungi and fungal toxins as weapons: currently the biggest concern is as a chronic health risk

Aflatoxins, Ochratoxin, citrinin, ergot alkaloids, patulin, fumonisins, trichothecenes

".. endemic in cornfields around the world." Fungal toxins are poisoning Africa's children, says new report By Catherine Matacic, Feb. 18, 2016 , Science (http://www.sciencemag.org/news/2016/02/fungal-toxins-are-poisoning-africa-s-children-says-new-report)

Rates of stunting among children under 5 are as low as 2.1% in the United States and as high as 59.3% in Afghanistan. (Data from the World Health Organization)



Antibiotic / Fungicide Resistance

Antibiotics provide treatment for bacterial infections

Antibiotic resistance has become a serious concern as it lowers or eliminates treatment options

Antifungals provide treatment for fungal infections

Antifungal resistance is now a major concern with *Candida* infections and many plant pathogens

Implication – infections with resistance cannot be cured

Canadian Definition of Terrorist Activity

The *Criminal Code* defines terrorist activity to include an act or omission undertaken, in or outside Canada, for a political, religious or ideological purpose, that is intended to intimidate the public with regard to its security, including its economic security, or to compel a person, government or organization (whether in or outside Canada) to do or refrain from doing any act, and that intentionally causes one of a number of specific forms of serious harm.

Canada has a history of terrorist attacks

Early 1960s - Sons of Freedom, extreme Doukhobor sect in British Columbia. "Over about four years, the group blew up railway tracks, hydro towers and other industrial and government targets in the province."

The 1970s - October Crisis precipitated by the Front de libération du Québec (FLQ) kidnapped and killed a provincial cabinet minister and kidnapped a British diplomat

1985 bombing of Air India flight 182 that claimed 329 lives.

From: http://www.ottawacitizen.com/news/canada-in-afghanistan/Terror+risk+Canada+lowest+among+major+western+economies+study/5207439/story.html#ixzz1eXDZsUkX

Canada and Terrorists

Terrorism Incidents 2017

- Jan. 29, 2017: Six dead and eight injured after shooting incident at a Quebec City mosque
- Aug. 10, 2016: Police shoot and kill terror suspect Aaron Driver in Strathroy, Ont.
- Oct. 22, 2014: Parliament Hill security and police shoot and kill Michael Zehaf-Bibeau after he killed Canadian soldier Cpl. Nathan Cirillo at the National War Memorial and then stormed the Parliament Buildings.
- FROM: http://globalnews.ca/news/3213312/canada-terrorist-incidents/

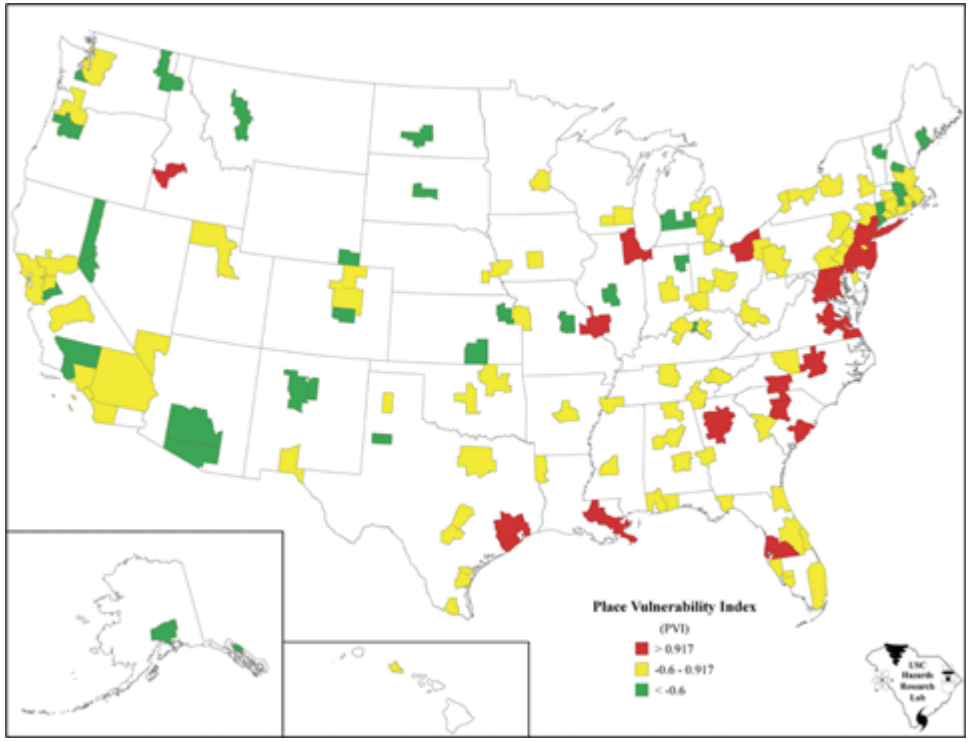
Societal risks in Canada

- Assessments usually considered for terrorist attacks or acts of war
- Risk results from a combination of an asset, a threat/hazard, and a vulnerability
  - All three elements must be present
  - If any element is missing, there is no risk

Threat Assessment

Determine which infrastructure is key to society and assess how the functioning of this infrastructure may be compromised

E.g. Energy and utilities, communication and information technology, heath care, policing, government, transportation etc.



Why use Biological Agents

Relatively easy to obtain

Relatively inexpensive to produce, store and transport,

Difficult to detect.

Effect can be hours or days later; therefore, identifying the attacker is very difficult

Spread via air, water, food or a human being

Some agents spread from human to human (e.g. small pox) while others do not (e.g. anthrax)

Biological Agent Attack on a City of 1,000,000 people (WHO estimates)

Disease Caused by Agent	Number of People at risk	Deaths	Incapacitation
Anthrax	180,000	95,000	30,000
Brucellosis	100,000	400	79,600
Epidemic typhus	100,000	15,000	50,000
Plague	100,000	44,000	36,000
Q fever	180,000	150	124,850
Tularemia	180,000	30,000	95,000
Venezuelan equine encephalitis	60,000	200	19,8000

Carus 2002

Force Multiplying Effect

Anthrax Letter attack in the US

- 3 teaspoons worth of anthrax
- $27,500,000 to clean up Senate Office Building
- $300,000,000 to clean up postal facilities
- Two pounds of anthrax would saturate all of Manhattan – a city of ~ 1.6 million

Three Levels of Crime

Biological Crime

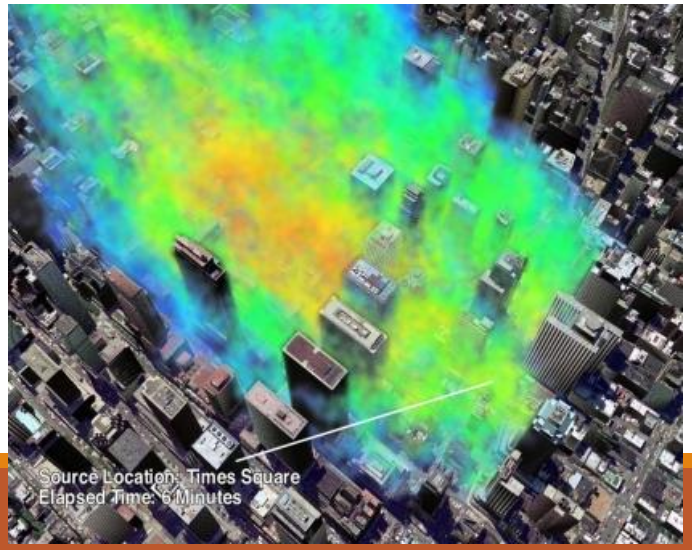
- Individuals or small group non-political and non-warfare driven

Biological terrorism

- Terrorist groups maybe nation supported
- https://emergency.cdc.gov/bioterrorism/

Biological Warfare

- Nation states, acts of war



Canada's Anti-Terrorism Act, 2015

Focused on stopping the promotion of terrorism

- Preventing terrorists from recruiting others
- Disrupting terrorist plots and preventing attacks
- Modified criminal code and ability of Law agencies to collect information
- https://www.justice.gc.ca/eng/cj-jp/ns-sn/act-loi.html
- Note on this web page the link to Bill C59: Our Security, Our Rights

Microbial Forensics


MALDI Biotyper

"a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime or inadvertent microorganism/toxin release for attribution purposes" Breeze et al 2005

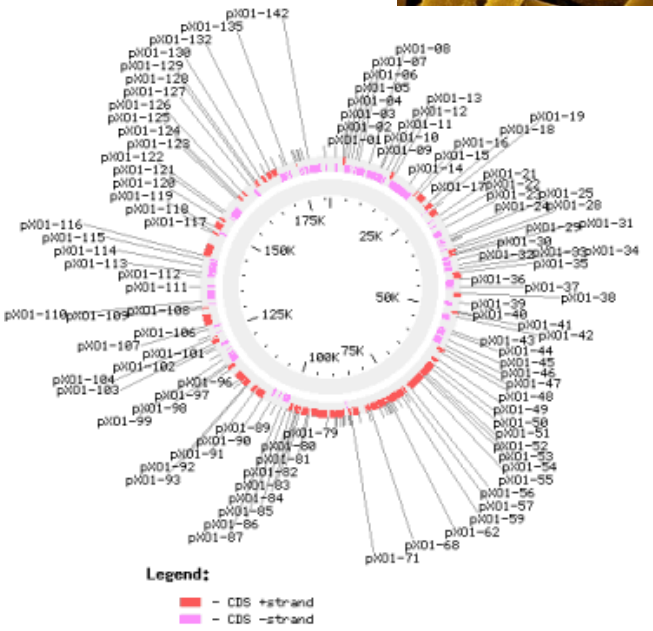
Unique identification difficult because of clonal reproduction

Most evidence is expected to fall into the class-characteristic category whether limited or informative

DNA Variability Among Agents

Low variability among some biocrime agents (e.g. *B. anthracis*) may allow I.D. to strain level but not sub strain, use combination MLVA and SNP

Viruses - high variability, ID via phylogenetic trees - similarity by descent i.e. those with the closest ancestors



Graphical representation of Bacillus anthracis virulence plasmid pX01, complete sequence. Credit: NCBI/NLM/NIH, Tatiana Tatusova

Non-DNA Methods

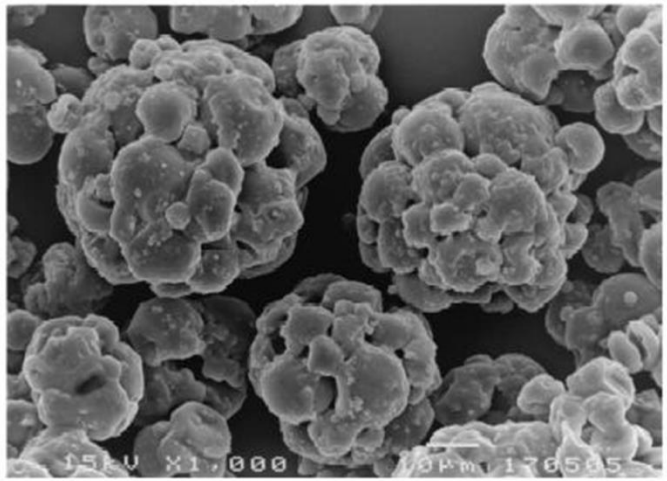
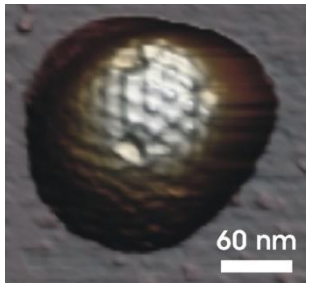


Fig. 5. Scanning electron micrograph of a spray-dried formulation containing 5% gelatinized tapioca starch, 5% sucrose, and 3% rice bran oil (formulation A) at 1,000× magnification.

Scanning electron microscopy,

- energy dispersive X-ray microanalysis



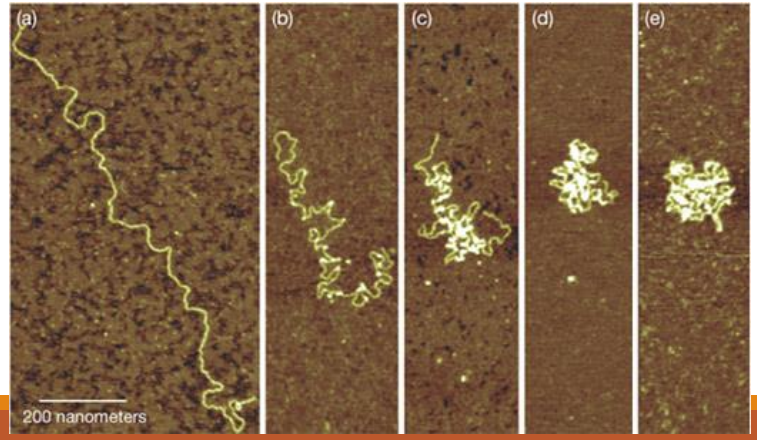
Atomic force microscopy

Raman spectroscopy and surface enhancement

Mass spectrometry including, bioaerosol time-of-flight and time-of-flight secondary ion

Nuclear Microscopy

Accelerator mass spectrometry



(a–e) Progressive images from atomic force microscopy show the compaction of DNA in yeast caused by a protein called AbF2.

Developing a Robust Microbial Forensics Program

In U.S., Bioforensics Analysis Center, BFAC,

- 1) Knowledge components
- 2) Strong Partnership Laboratory Network
- 3) Scientific Working Group on Microbial Genetics and Forensics (SWGMGF)

Review Bioterrorism

The biological agents
- Viruses, Bacteria
- Fungi, Biological toxins

Terrorism

Why biological agents

Microbial Forensics