Classification of Microorganisms (Chapter 10)

Lecture Materials

for

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Primary Source for figures and content:

Tortora, G.J. Microbiology An Introduction 8th, 9th, 10th ed. San Francisco: Pearson Benjamin Cummings, 2004, 2007, 2010.

Taxonomy = science of classification
- 1.7 million organisms identified so far
- estimated 10-100 million total on earth

All cellular organisms evolved from common ancestor:
- -similar plasma membrane
- -use ATP for energy
- -use DNA for genetic storage

Observed differences due to random mutation and natural selection (Theory of Natural Selection: Darwin 1859)

All organisms organized into taxonomic categories by relatedness

Systematics / Phylogeny = study of evolutionary history and relatedness of organisms

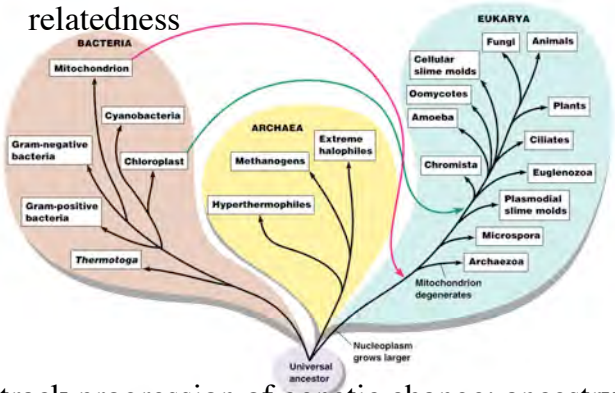
-originally classification based on appearance: arbitrary, many taxonomic schemes introduced

-modern taxonomy based on genetic sequence information (molecular biology)

rRNA sequences show three distinct groups:
1. eukaryotes (animals, plants, fungi, protists)
2. bacteria
3. archaea (prior to sequencing, Bacteria and Archaea had been grouped together in the kingdom Monera)

rRNA sequencing (1978) led to addition of Domain category to scientific nomenclature

Gene sequencing now allows for more accurate and precise placement of organisms into the taxonomic hierarchy of relatedness



(track progression of genetic change: ancestry)

Scientific Nomenclature

(binomial nomenclature)

-every organism has unique binomial that indicates the individual and its taxonomic placement among other organisms:

Genus: noun, capitalized

species / specific epithet: adjective

-whole name in italics, latinized

e.g. *Homo sapien* (= "man" "wise")

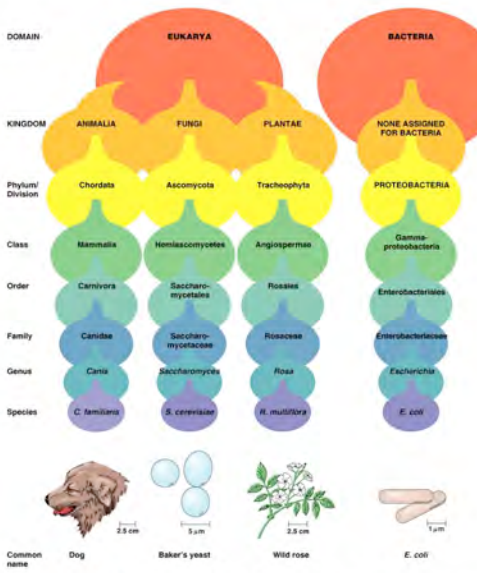
-when new organisms discovered, name must follow nomenclature rules and classify organism correctly in the taxonomic hierarchy:

Genus = group of species that differ from each other in certain ways but are related by descent

e.g. *Canis lupus* (wolf)
Canis latrans (coyote)
Canis aureau (jackal)
Canis familiaris (common dog)

Taxonomic / Phylogenetic Hierarchy:
-groups based on similarities
-begins very general, becomes more restricted
-DNA hybridization and rRNA sequencing used to determine evolutionary relationships and thus classification of each organism

Domain
Kingdom
Phylum
Class
Order
Family
Genus
Species



e.g. You (modern humans)

Domain	Eukarya	(all eukaryotes)
Kingdom	Animalia	(that are animals)
Phylum	Chordata	(with a backbone)
Class	Mammalia	(have hair, produce milk)
Order	Primate	(apes & monkeys)
Family	Hominidae	(great apes & human)
Genus	*Homo*	(all human ancestors)
Species	*sapien*	(modern man)

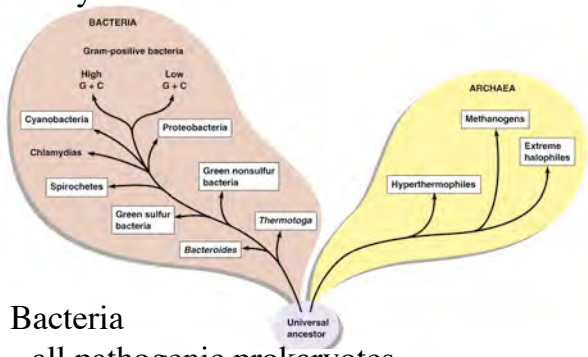
-organisms are grouped together based on relatedness: very general relatedness at the top, followed by more and more specific and restricted subgroups
genus = all related species
species = single unique organism group

Eukaryotic Classification
-all eukaryotes = domain eukarya
-four kingdoms:
1. Kingdom Protista (unicellular eukaryotes)
-algae and protozoa
-simple eukaryotes, don't fit elsewhere
-nutritionally diverse: autotrophs, heterotrophs, intracellular parasite, etc.
2. Kingdom Fungi
-yeasts, molds, mushrooms
-absorb organic material through plasma membrane
3. Kingdom Animalia
-multicellular animals
-ingest organic food through a mouth
-have cells organized into tissues
4. Kingdom Plantae
-multicellular plants
-undergo photosynthesis to convert CO_2 + H_2O into organic molecules
-have cells organized into tissues

Eukaryotic species = defined as a group of closely related organisms that can breed among themselves and produce fertile offspring
e.g.
horse, ass, and zebra are all in the genus *Equus*: each is different enough to be a separate species:
Equus caballus (horse)
Equus somalicus (ass)
Equus grevyi (zebra)
separate species usually cannot interbreed at all, if they can offspring are sterile:
horse X ass = mule (sterile)
Therefore, (repeated from above)
a eukaryotic species is defined as a group of closely related organisms that can breed among themselves and produce fertile offspring

Prokaryotic Classification

-prokaryotes = two domains:



1. Bacteria
   - -all pathogenic prokaryotes
   - -many non pathogenic prokaryotes
   - -all photoautotrophic prokaryotes
2. Archaea
   - -all prokaryotes with walls that are not peptidoglycan
   - -often carryout unusual metabolism and live in extreme environments

-no kingdoms, but all other taxonomic groups

-groupings based entirely on gene sequencing since most look similar

Prokaryotic species = defined as a population of cells with similar characteristics (no sexual reproduction)

Pure culture = clones, population derived from a single cell, genetically identical

Strains = cells of the same species that are not genetically identical in all ways

- -each culture or group that is slightly different is called a strain
- -each strain is indicated by a number or letter designation following the *Genus species* name

e.g. *Escherichia coli* - normal intestinal flora
Escherichia coli 0157:H7 -produces a toxin all other stains do not, deadly pathogen of humans

Viral Classification

-viruses do not fit domain system as they are acellular

-usually only classified by Family and Genus

-usually only referred to by common name

e.g. HIV (human immuno-deficiency virus)
Genus = *Lentivirus*
Family = Retroviridae

Viral species = defined as a population of viruses with similar characteristics (including morphology, genes and enzymes) that occupy a particular ecological niche

-viruses are obligate intracellular parasites: they evolved to infect cells

-they usually only infect one type of cell: the one that best supports the viral replication

-thus viruses tend to be very specific about their niche:

e.g. HIV: infects only human T helper cells

Identification of Microorganisms

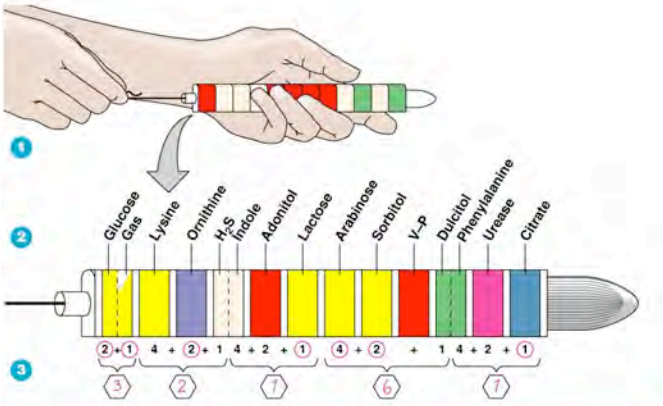
-classification into taxonomic hierarchy based on morphological characteristics, DNA hybridization, and rRNA sequencing

-identification of an unknown (but previously discovered and classified) microbe requires more specific and often combined methods

1. Morphological characteristics
   - -size, shape, cellular characteristics (capsule, flagella, endospores, etc.)
2. Differential staining
   e.g. Gram stain, Acid fast stain
3. Biochemical tests
   - -probe for specific enzyme activities:
     - -carbohydrate fermentation
     - -nitrogen fixation
     - -sulfur oxidation
     - -gas production
     - -acid production
     - -nitrate reduction
     - -etc.

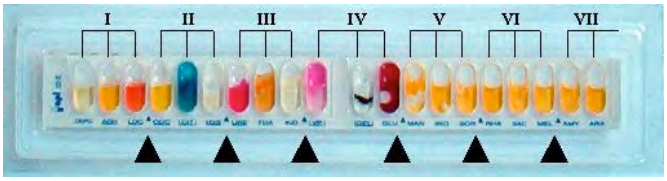
-rapid determination tools:
  a. selective media: inhibits the growth of one group while allowing another to flourish
    e.g. salt tolerance broth: selects for organisms that are tolerant of 6.5% NaCl (Staph and Streps)
  b. differential media: allows all organisms to grow but causes one group to appear different
    e.g. MacConkey agar: lactose fermenters turn pink
  c. multi-test systems/numerical ID
    e.g. Enterotube
      API test systems

Enterotube



ID Value	Organism	Atypical Test Results	Confirmatory Test
32143	*Enterobacter cloacae*	Sorbitol$^-$	–
	Enterobacter sakazakii	Urea$^+$	+
32161	*Enterobacter cloacae*	None	V–P$^+$
32162	*Enterobacter cloacae*	Citrate$^-$	

API

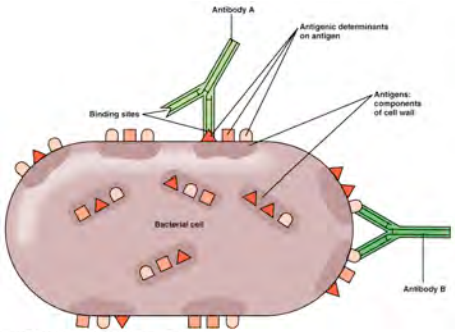


4. Serology

serology = science of serum and immune responses that are evident in serum (blood plasma w/o fibrinogen)

-involves use of antibodies to detect specific microbe antigens (proteins)

antibody = special protein, produced by animals, to bind to a specific target (its antigen, usually a protein)



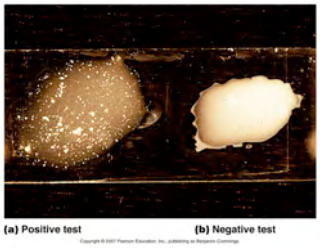
-the immune response of an animal can produce antibodies to any molecule (antigen) that is foreign to that animal

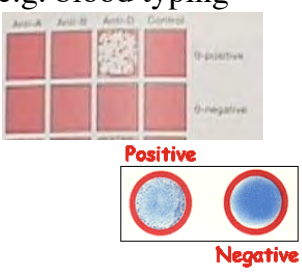
-diagnostic antibodies can be produced to detect particular microbes:
  1. inject animal with microbe
  2. allow immune response (1-2 weeks)
  3. harvest blood
  4. purify out antibodies

antiserum = a solution that contains purified antibodies against a particular antigen (or microbe)

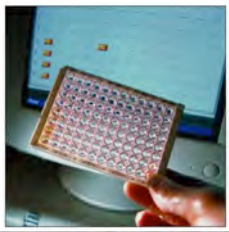
Antiserum to known antigens can be used to identify the antigen in an unknown sample:

A. Agglutination tests
  specific antibody + its antigen = clumps
  (clumping = agglutination: antibody bound to antigen) e.g. blood typing

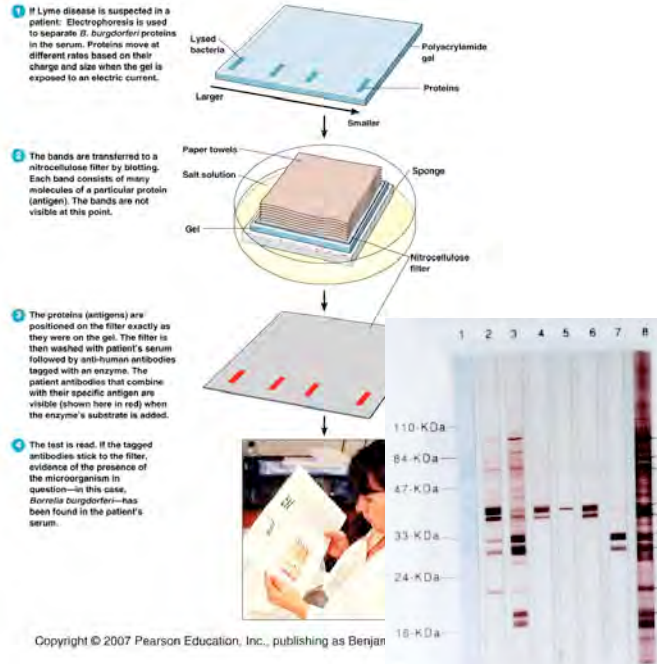




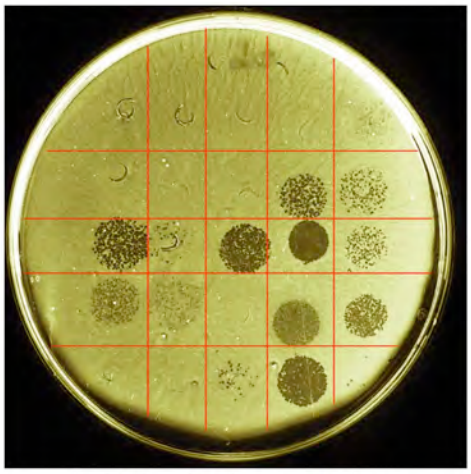
B. ELISA
(enzyme linked immunosorbent assay)
antibody + antigen = color change
-96 reactions at a time in microtiter dishes
-can be automated
-rapid, but risk false positives
e.g. rapid HIV, pregnancy



(a)

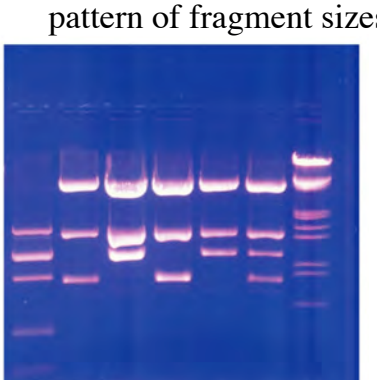
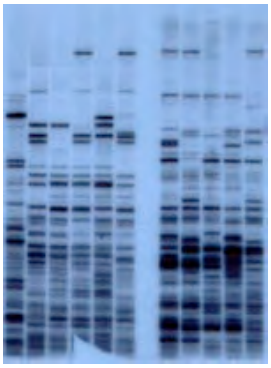

C. Western Blot
antibody + antigen = color change on blot
-more precise and accurate than ELISA but more time consuming, no automation
e.g. HIV conformation, Lyme disease



5. Phage typing / Plaque assay
Phage = bacterial virus
-each phage is very specific: infects only one species or even strain of bacteria
-when phage infects, it causes lysis of the bacteria
-apply known phages to a lawn of the unknown bacteria and look for bacterial cell death: clear zone = plaque



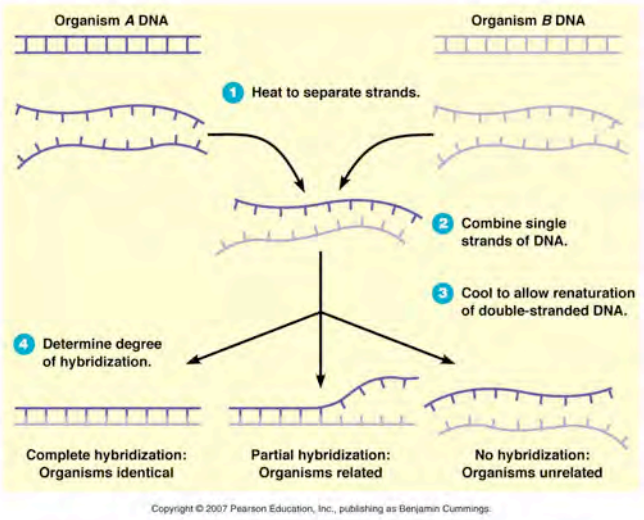
6. DNA Sequence Methods
-identify based on unique nucleotide base sequence in the chromosomal DNA
A. DNA fingerprinting/RFLP Analysis
-use restriction enzymes to cut the chromosomal DNA at specific known sequences (each enzyme specific for one sequence e.g. EcoRI cuts GAATTC)
-resulting fragments of DNA are separated by size via gel electrophoresis
-since genomic sequences vary with each species and strain, each produces a unique pattern of fragment sizes

B. PCR (Polymerase Chain Reaction)
- "DNA photocopying"
-allows tiny amounts of DNA to be replicated specifically out of a sample
-identify species or strain by DNA sequence of a particular gene, or presence of some unique gene or DNA segment

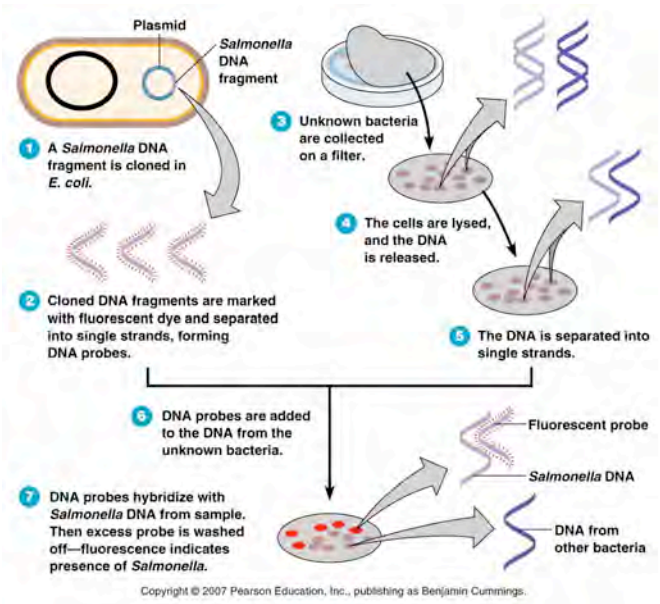
7. Nucleic Acid Hybridization
-heat DNA to separate the strands (break H-bonds between complementary bases)
-when cooled double helix will reform by complementary base pairing
-actual direct sequencing can be time consuming, not practical for large DNA
-hybridization can more rapidly determine the similarity of two sequences (for relatedness of two species)

-heat DNA of organisms to be analyzed
-mix, cool, allow to anneal (complementary base pair)
-assess degree of hybridization

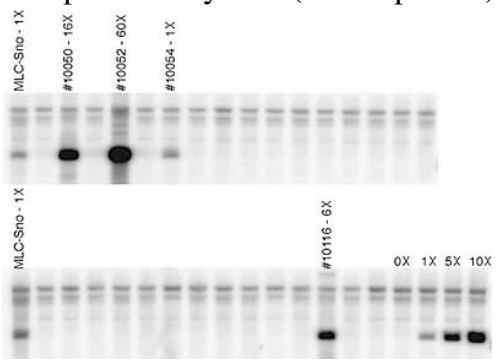


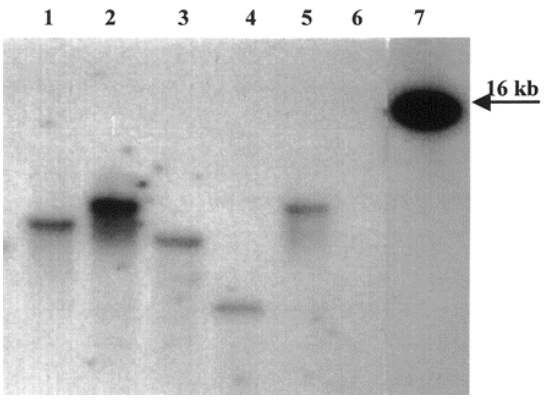
-the more hybridization, the more similar the DNA sequence, the greater the degree of relatedness

A. Southern Blot
-probe suspect samples with single stranded, dye-labeled known DNA
-complete hybridization (exact sequence match) results in visible color
-can be performed on bacterial colonies



-or DNA samples separated on gel electrophoresis by size (more specific)





B. DNA Chips

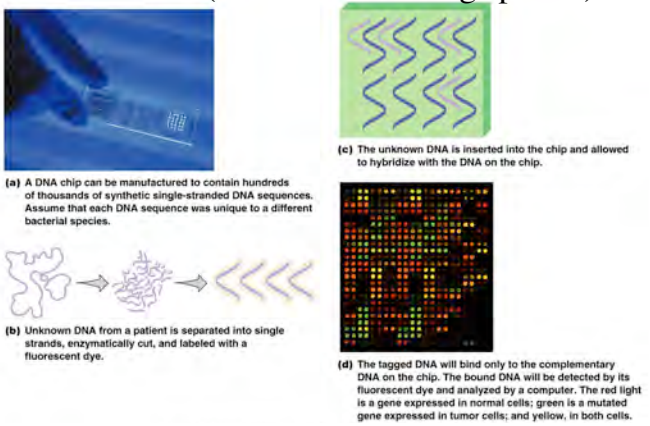
-chip contains single stranded DNA probes (e.g. library of all viruses, library of all *E.coli* strains, etc.)

-add patient sample to chip

-binding of matching sequences causes color change

-colors detected by computer and scored for intensity

-read out indicates identity of probe that bound best (identifies matching species)



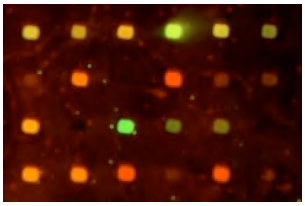
(a) A DNA chip can be manufactured to contain hundreds of thousands of synthetic single-stranded DNA sequences. Assume that each DNA sequence was unique to a different bacterial species.

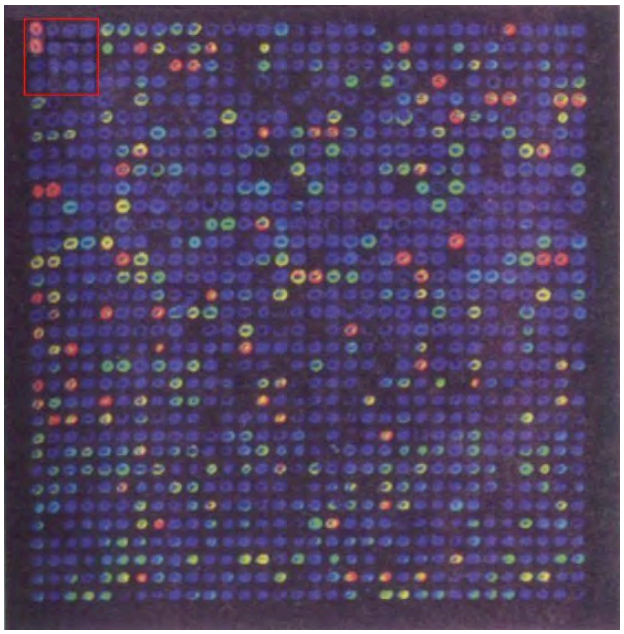
(b) Unknown DNA from a patient is separated into single strands, enzymatically cut, and labeled with a fluorescent dye.

(c) The unknown DNA is inserted into the chip and allowed to hybridize with the DNA on the chip.

(d) The tagged DNA will bind only to the complementary DNA on the chip. The bound DNA will be detected by its fluorescent dye and analyzed by a computer. The red light is a gene expressed in normal cells; green is a mutated gene expressed in tumor cells; and yellow, in both cells.

DNA Chip



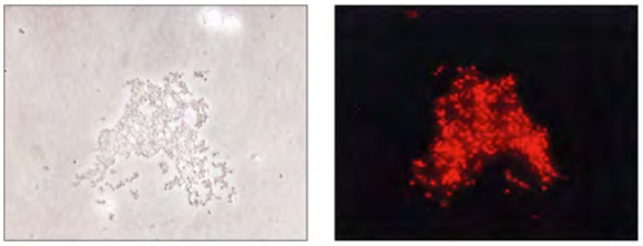


C. FISH (Fluorescent In Situ Hybridization)

-fluorescent dye labeled DNA probes are added to mixed sample (e.g. biopsy, environment sample, etc.)

-hybridization "tags" cells with that sequence

-cells are observed using UV light



(a) (b) 10 μm