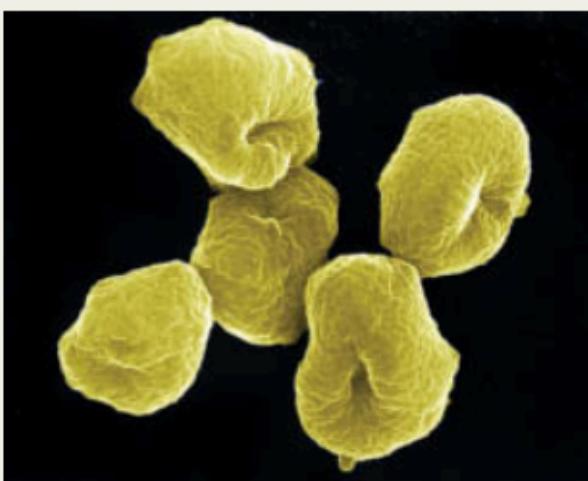


MIKROBIOLOGI

PERTEMUAN KE 3
IRMA MARDIAH M.SI

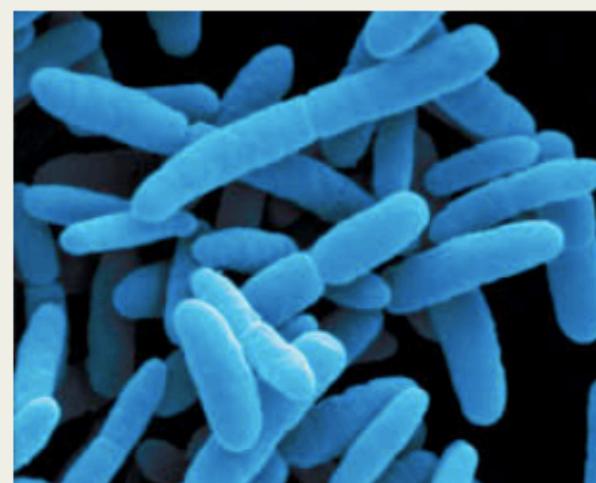
Archaea



Sulfolobus sp.

SEM
1 μm

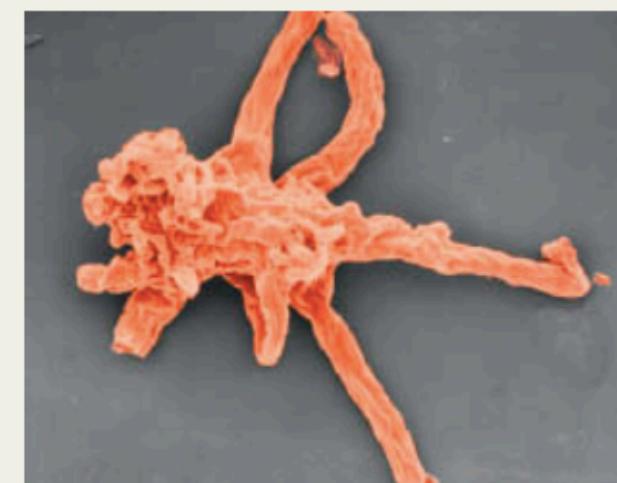
Bacteria



E. coli

SEM
1 μm

Eukarya



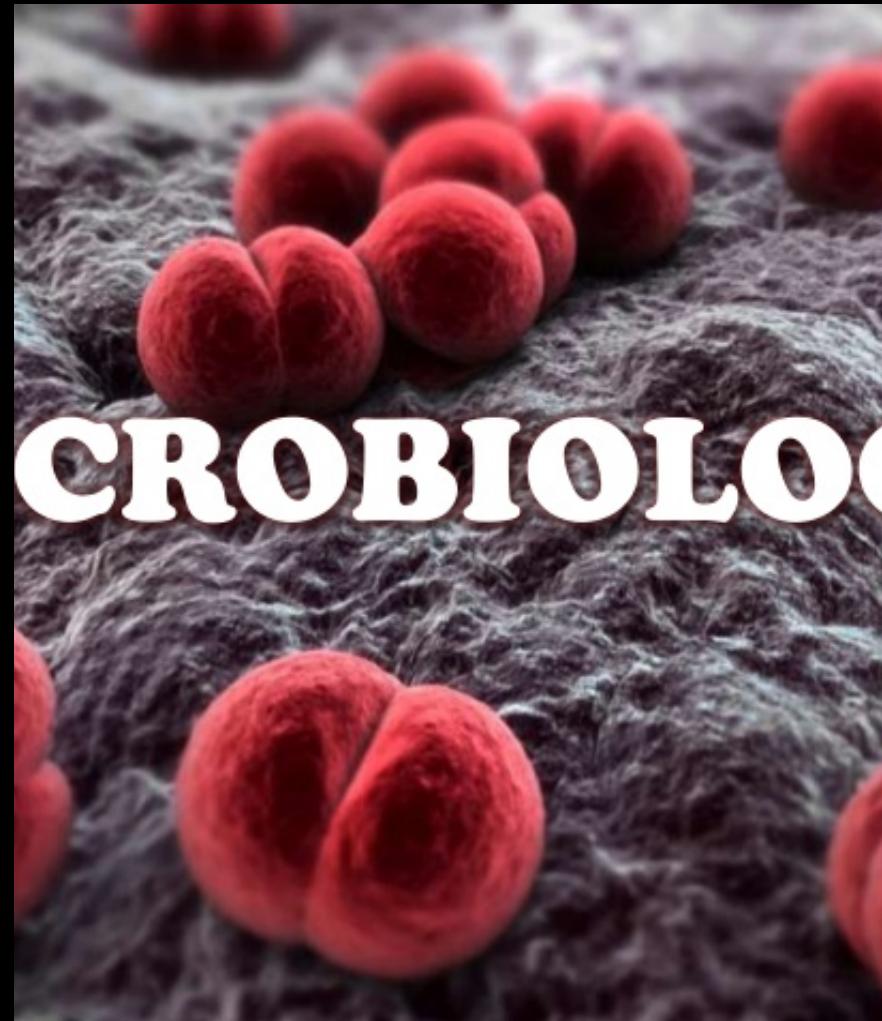
Amoeba sp.

SEM
5 μm

KLASIFIKASI MIKROORGANISME TAKSONOMI?

KONSEP KUNCI

- Studi hubungan filogenetik
- Klasifikasi Organisme
- Metode Klasifikasi & Identifikasi Mikroorganisme



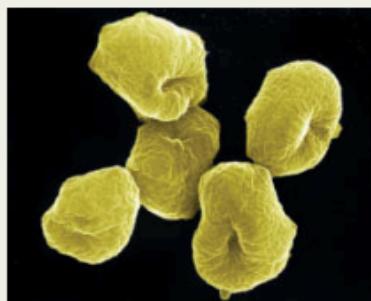
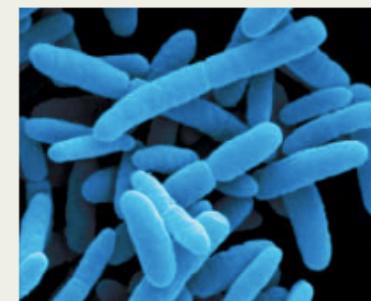
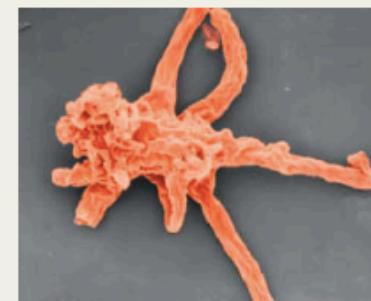
Studi hubungan Filogenetik

- taksonomi, taxon & filogeni
- diskusi keterbatasan klasifikasi dua kongdom
- identifikasi kontribusi Linnaeus, Whittaker, dan Woese
- diskusi keuntungan tiga domain
- daftar karakteristik Bakteri, Archaea dan domain Eukarya



Tiga Domain

TABLE 10.1 Some Characteristics of Archaea, Bacteria, and Eukarya

Archaea	Bacteria	Eukarya
		
Sulfolobus sp. SEM 1 μm	E. coli SEM 1 μm	Amoeba sp. SEM 5 μm
Cell Type	Prokaryotic	Eukaryotic
Cell Wall	Varies in composition; contains no peptidoglycan	Contains peptidoglycan
Membrane Lipids	Composed of branched carbon chains attached to glycerol by ether linkage	Composed of straight carbon chains attached to glycerol by ester linkage
First Amino Acid in Protein Synthesis	Methionine	Formylmethionine
Antibiotic Sensitivity	No	Yes
rRNA Loop*	Lacking	Present
Common Arm of tRNA†	Lacking	Present

*Binds to ribosomal protein; found in all bacteria.

†A sequence of bases in tRNA found in all eukaryotes and bacteria: guanine-thymine-pseudouridine-cytosine-guanine.

Pohon Filogenetik



- struktur banyak mikroorganisme tidak terfosilisasi, kecuali beberapa hal berikut:
 - protista laut yang terfosilisasi-koloni dari White Cliffs of Dover, Inggris
 - stromatolites, fosil dari bakteri filamen dan sedimen yang berkembang antara 0.5 dan 2 miliar tahun yang lalu
 - fosil seperti sianobakter ditemukan pada batu berusia 3-3.5 miliar tahun. ini dipercaya fosil tertua.

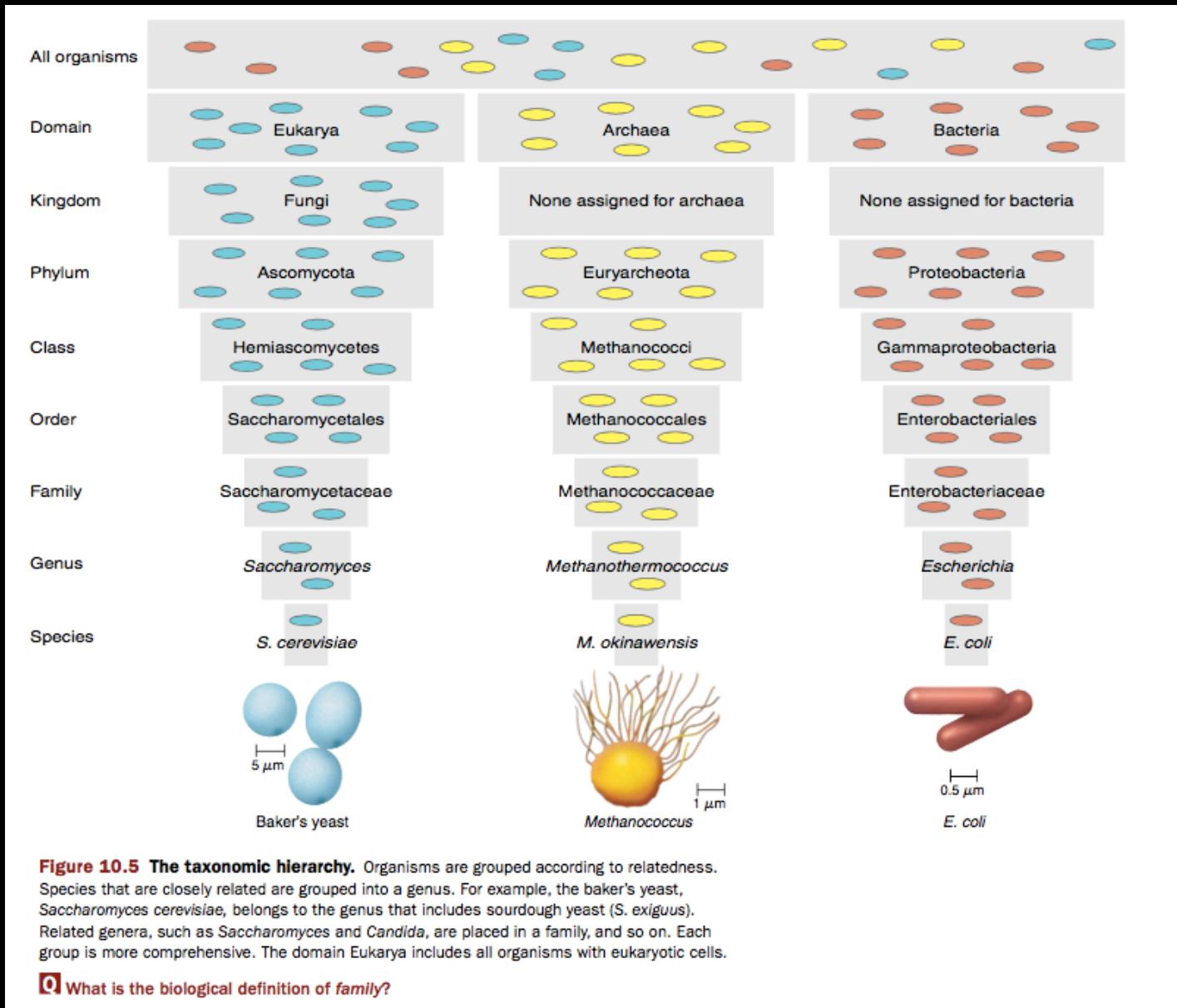
Klasifikasi Organisme

- Menjelaskan mengapa nama ilmiah digunakan
- daftar taxa ufana
- perbedaan kuur, kon dan strain
- daftar karakteristik utama yang digunakan untuk membedakan tiga kingdom multiselular eukarya
- protista
- membedakan eukaryot, prokaryot, dan spesies virus

Nomenklatur Ilmiah

Binomial nomenklatur: genus penanda spesies

Hirarki Taksonomi



Klasifikasi

- Eukarya
- prokariot
- virus

Metode Klasifikasi & Identifikasi Mikroorganisme

- perbandingan dan perbedaan klasifikasi dan identifikasi
- penjelasan tujuan manual Bergey's
- menggambarkan bagaimana pewarnaan & uji biokimia digunakan untuk mengidentifikasi bakteri
- perbedaan Western blotting dan Southern blotting
- menjelaskan bagaimana uji serologi dan tipe fage dapat digunakan untuk mengidentifikasi bakteri yang tidak diketahui
- menggambarkan bagaimana penemuan terbaru mikroba dapat diklasifikasikan dengan DNA sekuensing, DNA fingerpint, dan PCR
- menggambarkan bagaimana micro-organisme data diidentifikasi dengan hibridisasi asam nukleat, Southern Blotting, DNA child, robotsping dan FISH
- perbedaan Kunci dikotomi dari kladogram

Karakter Morfologi

digunakan selama lebih dari 200 tahun untuk
mengidentifikasi organisme

Tahap Pewarnaan

- Gram positif
- Gram negatif

Uji Biokimia

CLINICAL FOCUS Mass Deaths of Marine Mammals Spur Veterinary Microbiology

Over the past 20 years, thousands of marine mammals have died unexpectedly all over the world from a variety of infectious diseases. Notable outbreaks and problems include:

- The deaths of over 500 bottlenose dolphins along the mid-Atlantic coast due to *Brucella* spp. during 2010–2013
- The decline of the California sea otter population in recent years, with toxoplasmosis and other bacterial species responsible for a 40% mortality rate
- The deaths of over 100 harbor seals along the New England coast in 2011 due to influenza A H3N8
- The 2013 deaths of hundreds of bottlenose dolphins in the Atlantic

Ocean from cetacean morbillivirus, likely transmitted to dolphins from pilot whales.

- Large numbers of pathogens, including *Nocardia* and *Arcanobacterium*, have been identified in stranded dolphins, harbor seals, and sea otters

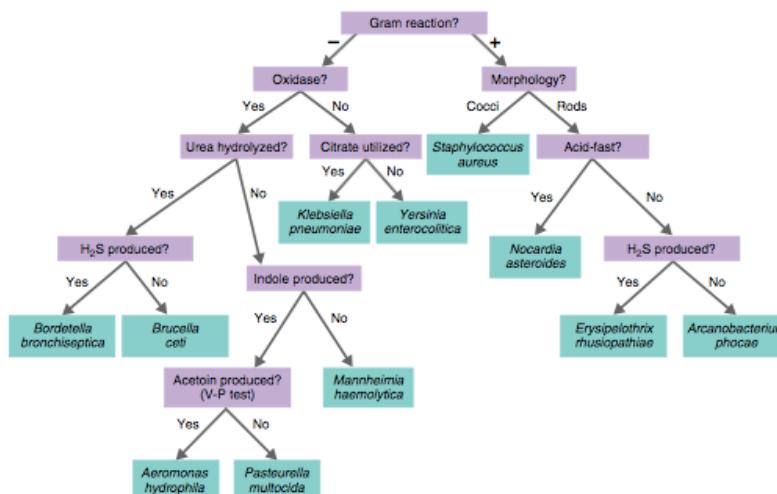
Information Is Scarce

Such issues are the concern of veterinary microbiology, which until recently has been a neglected branch of medical microbiology. Although the diseases of cattle, chickens, and mink have been studied, partly because of their availability to researchers, the microbiology of wild animals, especially marine mammals, is a relatively new field. Gathering samples from animals that live in the open ocean and performing bacteriological analyses on them is

very difficult. The animals being studied are those that have been stranded and those that come onto the shore to breed, such as the northern fur seal lion.

Microbiologists are identifying bacteria in marine mammals by using conventional test batteries (see figure) and genomic data of known species. The FISH technique is being used to find new species of bacteria in marine mammals (see page 288).

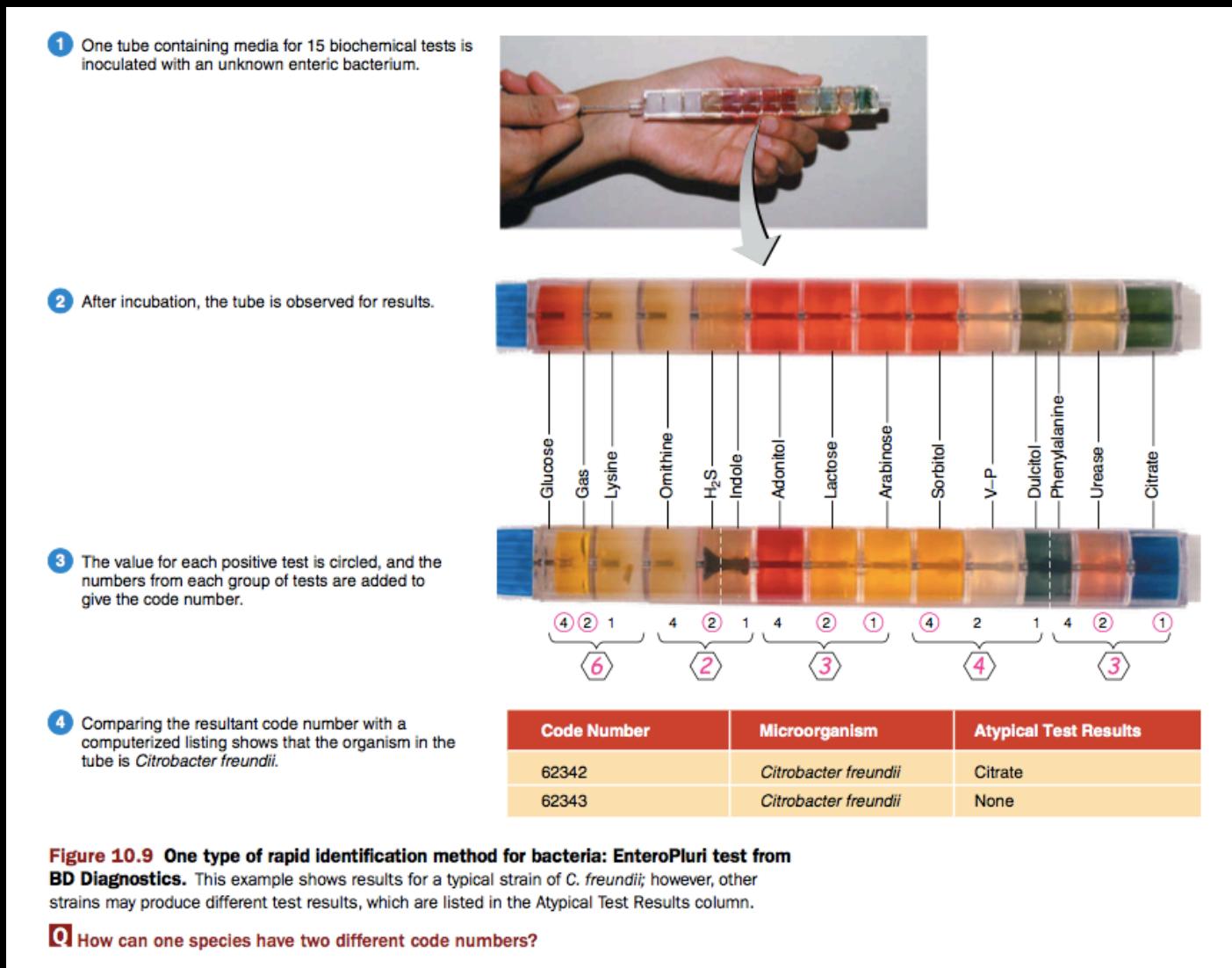
Veterinary microbiologists hope that studying the microbiology of wild animals, including marine mammals, will improve wildlife management and also provide models for studying human diseases.



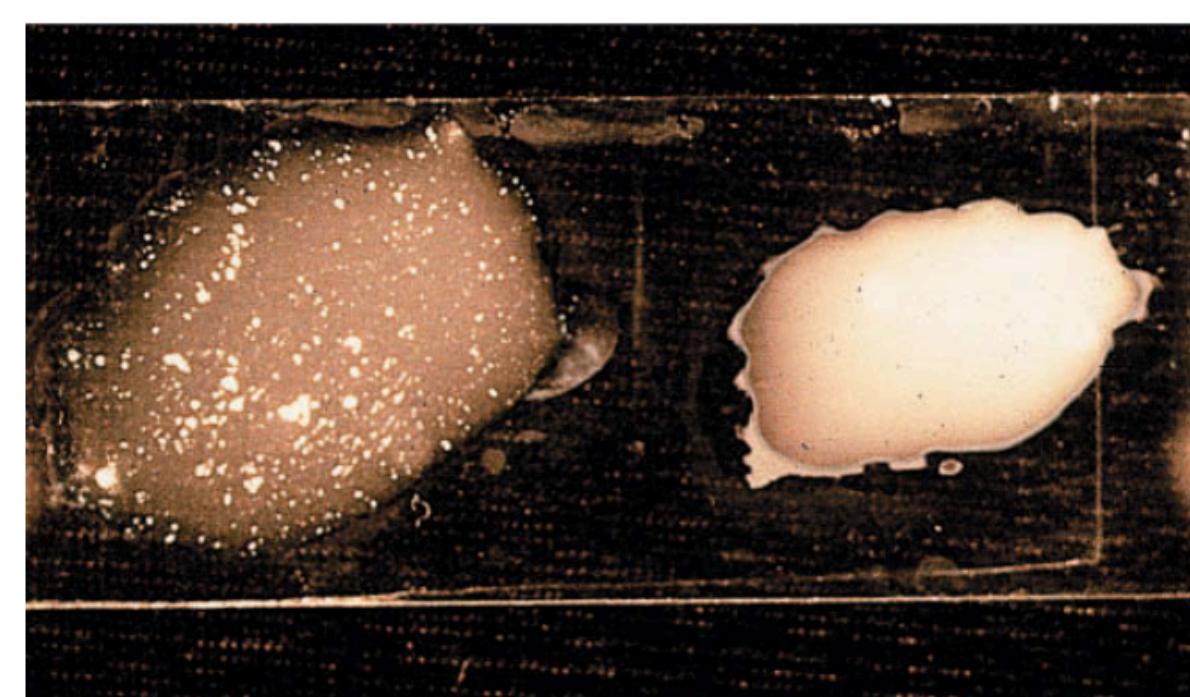
Biochemical tests used to identify selected species of human pathogens isolated from marine mammals.

Q Assume you isolated a gram-negative rod that is oxidase-positive, is indole-negative, and does not produce urease or acetoin. What is the bacterium?

Rapid Identification Method



Serologi



(a) Positive test

(b) Negative test

Figure 10.11 A slide agglutination test. (a) In a positive test, the grainy appearance is due to the clumping (agglutination) of the bacteria. (b) In a negative test, the bacteria are still evenly distributed in the saline and antiserum.

Q Agglutination results when the bacteria are mixed with _____.

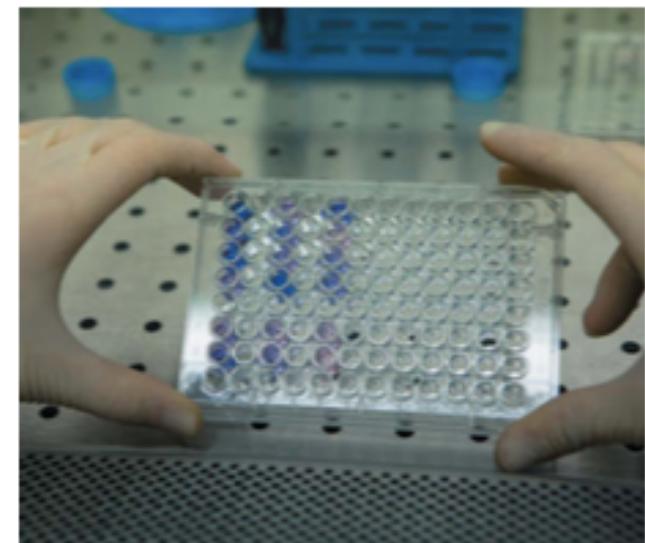
uji ELISA (Enzyme-Linked Immunosorbent Assay)

Figure 10.12 An ELISA test.

Q What are the similarities between the slide agglutination test and the ELISA test?



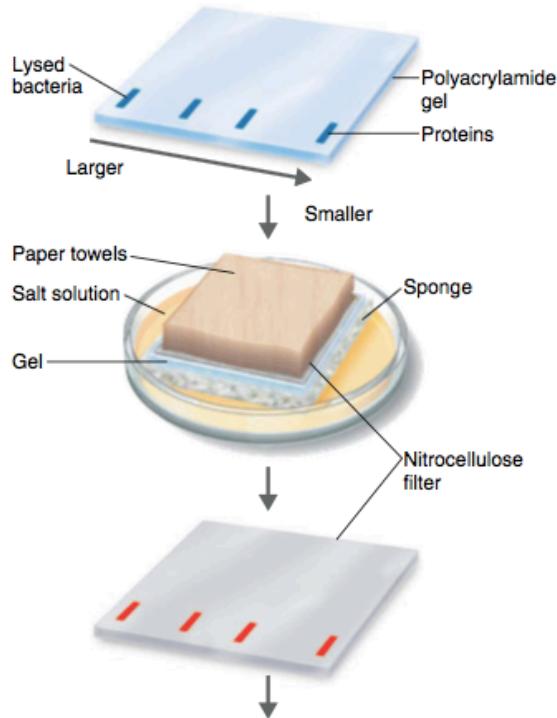
(a) A technician uses a micropipette to add samples to a microplate for an ELISA.



(b) ELISA results are then read using a spectrophotometer.

Western Blot

- 1 If Lyme disease is suspected in a patient: Electrophoresis is used to separate *Borrelia burgdorferi* proteins. Proteins move at different rates based on their charge and size when the gel is exposed to an electric current.



- 2 The bands are transferred to a nitrocellulose filter by blotting. Each band consists of many molecules of a particular protein (antigen). The bands are not visible at this point.

- 3 The proteins (antigens) are positioned on the filter exactly as they were on the gel. The filter is then washed with patient's serum followed by anti-human antibodies tagged with an enzyme. The patient antibodies that combine with their specific antigen are visible (shown here in red) when the enzyme's substrate is added.

- 4 The test is read. If the tagged antibodies stick to the filter, evidence of the presence of the microorganism in question—in this case, *B. burgdorferi*—has been found in the patient's serum.

Figure 10.13 The Western blot. Proteins separated by electrophoresis can be detected by their reactions with antibodies.

Q Name two diseases that may be diagnosed by Western blotting.

Phage Typing

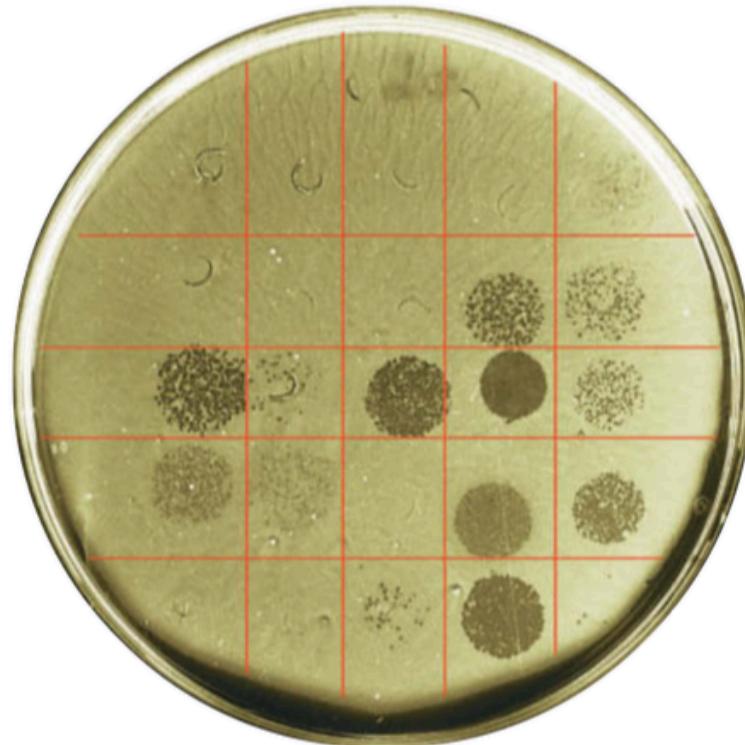


Figure 10.14 Phage typing of a strain of *Salmonella enterica*. The tested strain was grown over the entire plate. Plaques, or areas of lysis, were produced by bacteriophages, indicating that the strain was sensitive to infection by these phages. Phage typing is used to distinguish *S. enterica* serotypes and *Staphylococcus aureus* types.

Q What is being identified in phage typing?

Flow Cytometry

DNA Sequencing

DNA Fingerprinting

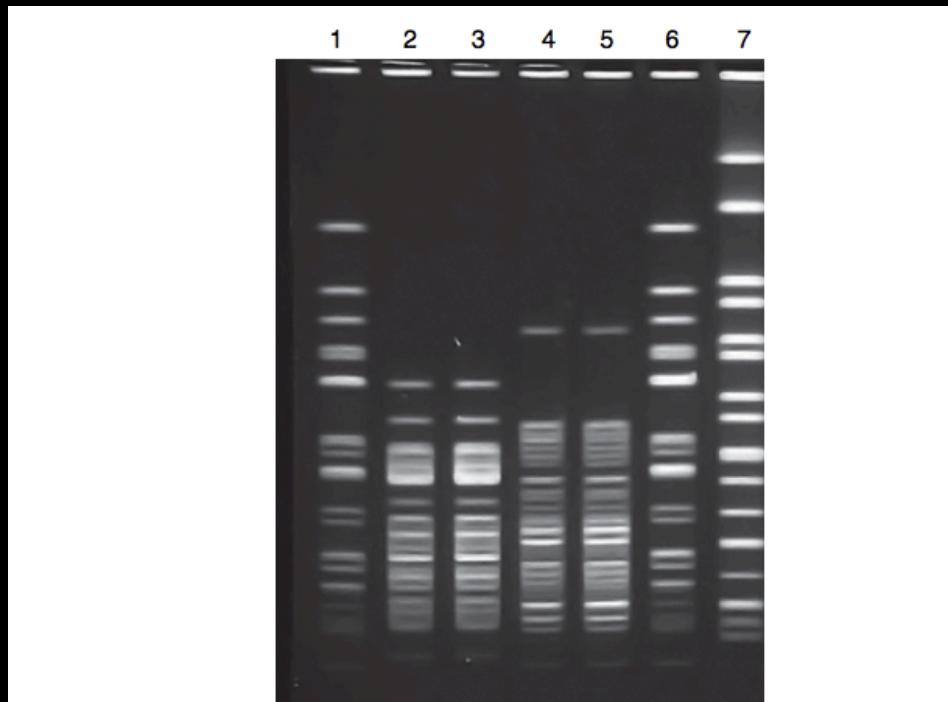
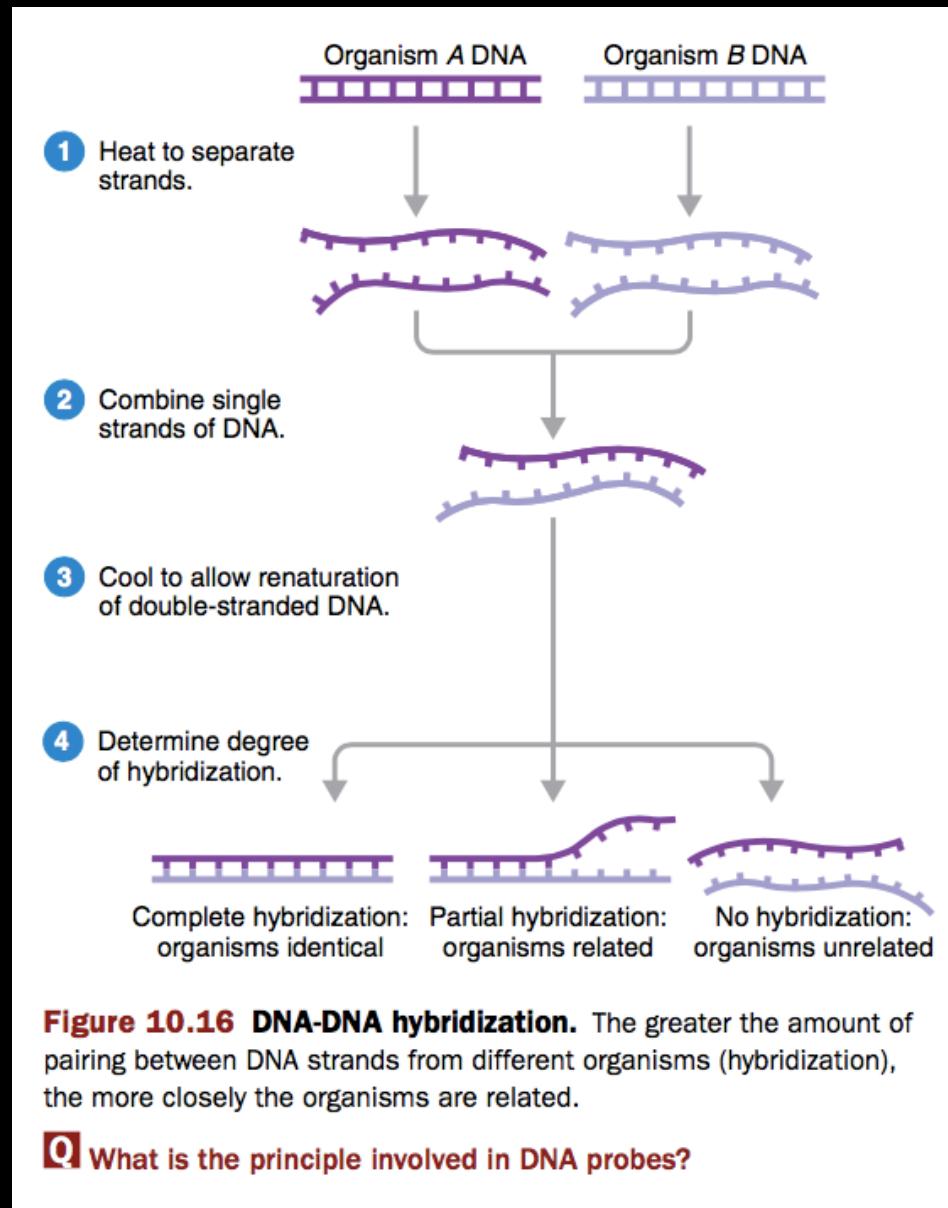


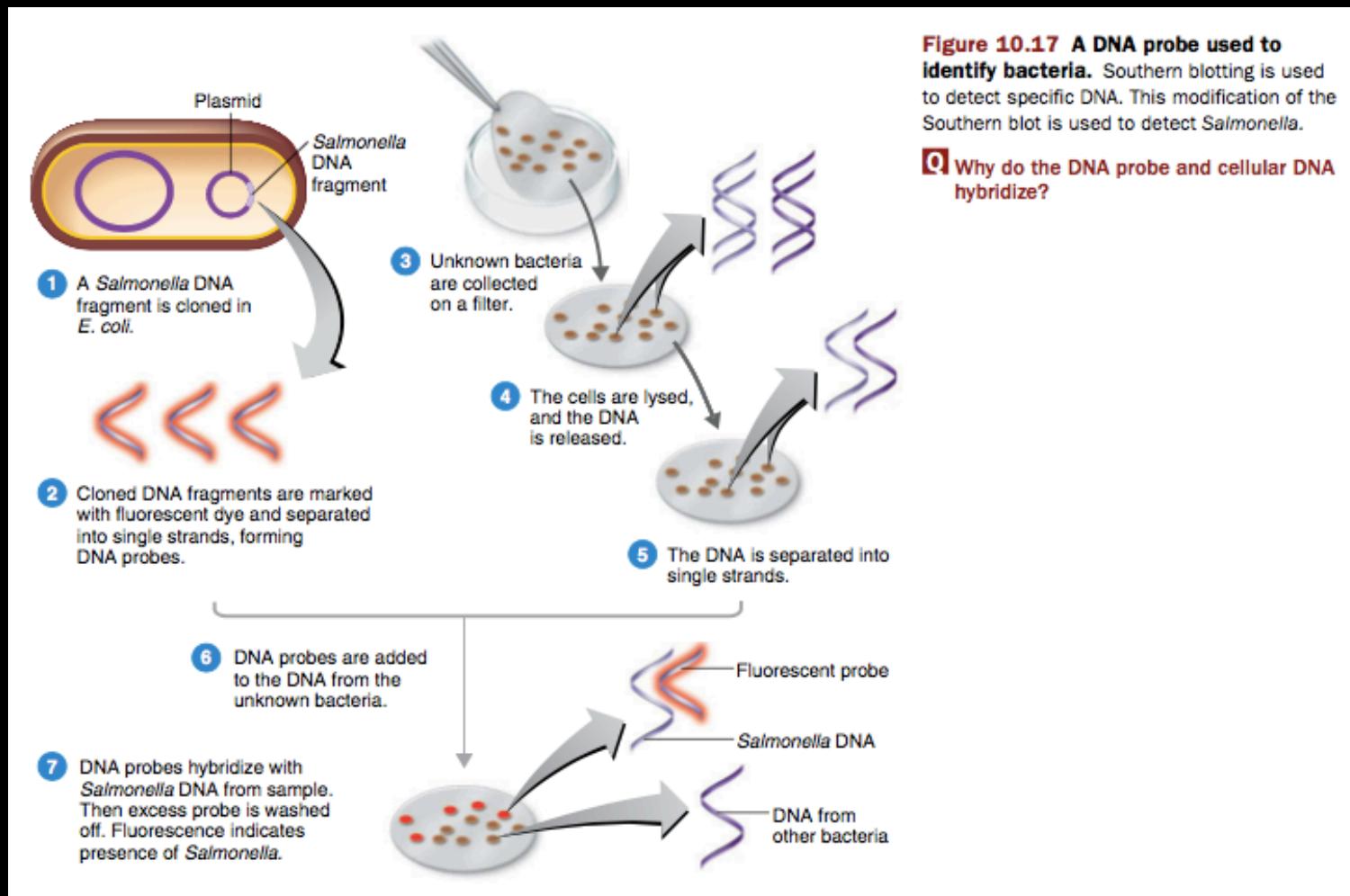
Figure 10.15 DNA fingerprints. DNA from seven different bacteria was digested with the same restriction enzyme. Each digest was put in a different well (origin) in the agarose gel. An electrical current was then applied to the gel to separate the fragments by size and electrical charge. The DNA was made visible by staining with a dye that fluoresces under ultraviolet light. Comparison of the lanes shows that DNA samples (and therefore the bacteria) in lanes 2 and 3; 4 and 5; and 1 and 6 are identical.

Q Differentiate between a gene and an RFLP.

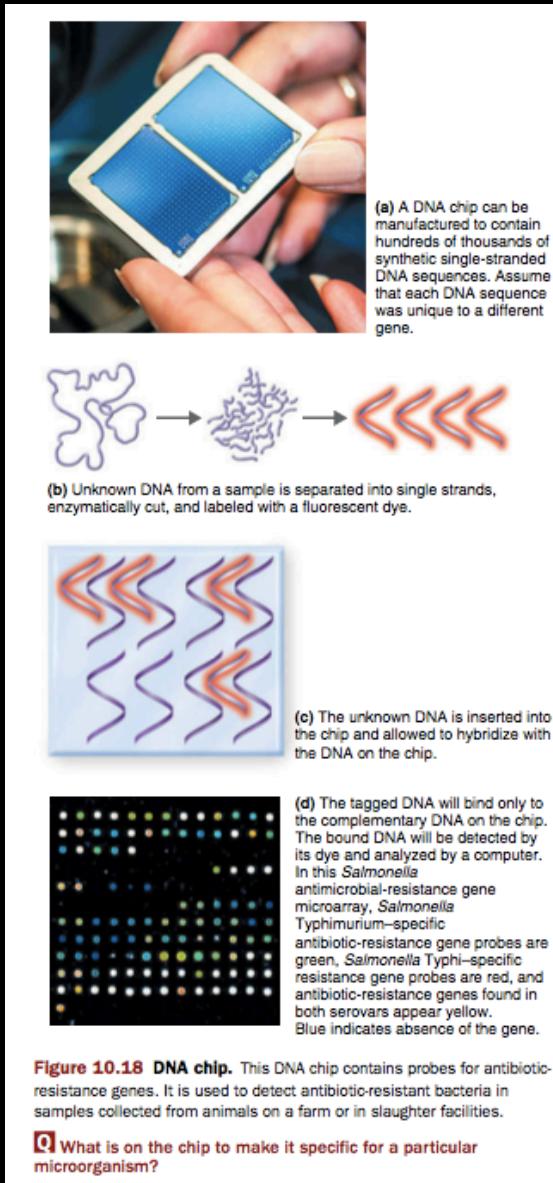
Hibridisasi Asam Nukleat



Southern Blotting



DNA Chips



FISH (Fluorescent In Situ Hybridization)

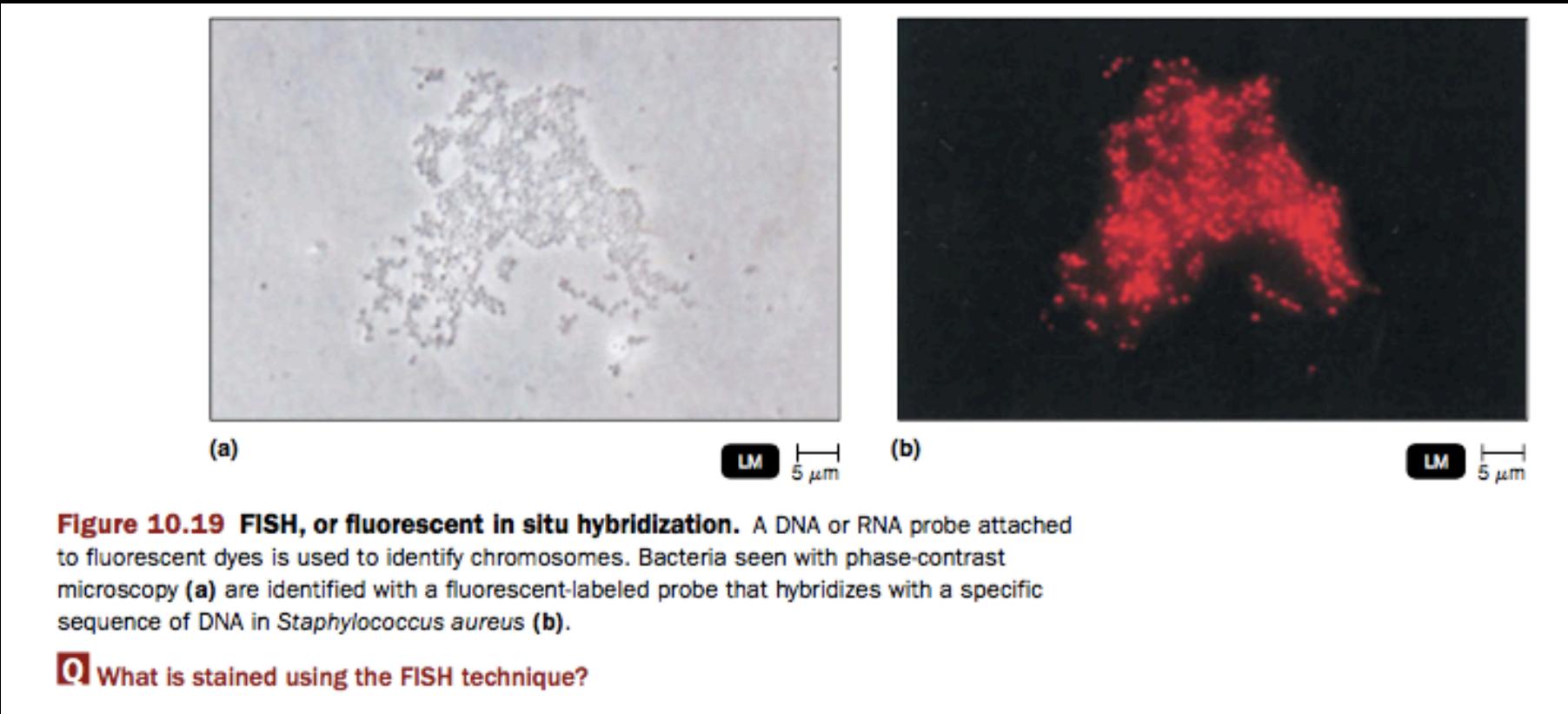


Figure 10.19 FISH, or fluorescent in situ hybridization. A DNA or RNA probe attached to fluorescent dyes is used to identify chromosomes. Bacteria seen with phase-contrast microscopy (a) are identified with a fluorescent-labeled probe that hybridizes with a specific sequence of DNA in *Staphylococcus aureus* (b).

Q What is stained using the FISH technique?

Kladogram

- 1 Determine the sequence of bases in an rRNA molecule for each organism. Only a short sequence of bases is shown for this example.

<i>Lactobacillus brevis</i>	AGUCCAGAGC
<i>L. sanfranciscensis</i>	GUAAAAGAGC
<i>L. acidophilus</i>	AGCGGAGAGC
<i>L. plantarum</i>	ACGUUAGAGC

- 2 Calculate the percentage of similarity in the nucleotide bases between pairs of species. For example, there is a 70% similarity between the sequences for *L. brevis* and *L. acidophilus*.

	Percent similarity
<i>L. brevis</i> → <i>L. sanfranciscensis</i>	50%
<i>L. brevis</i> → <i>L. acidophilus</i>	70%
<i>L. brevis</i> → <i>L. plantarum</i>	60%
<i>L. sanfranciscensis</i> → <i>L. acidophilus</i>	50%
<i>L. sanfranciscensis</i> → <i>L. plantarum</i>	50%
<i>L. plantarum</i> → <i>L. acidophilus</i>	60%

- 3 Construct a cladogram. The length of the horizontal lines corresponds to the percent similarity values. Each branch point, or node, in the cladogram represents an ancestor common to all species beyond that node. Each node is defined by a similarity in rRNA present in all species beyond that branch point.

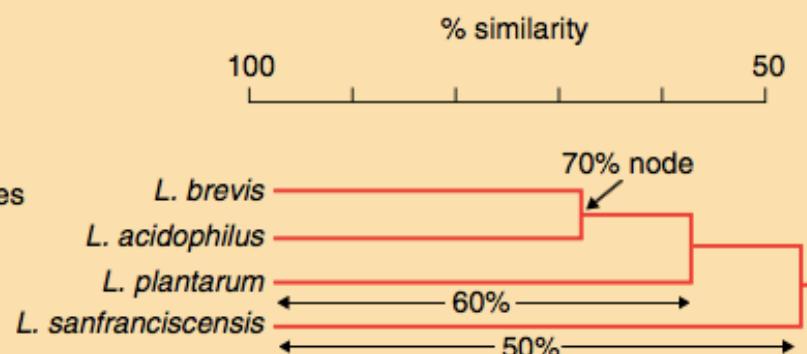


Figure 10.20 Building a cladogram.

Q Why do *L. brevis* and *L. acidophilus* branch from the same node?

EXPLORING THE MICROBIOME

Techniques for Identifying Members of Your Microbiome

In the past, the main way to identify a microbe from your microbiome—say, a bacterium in your intestines—would be to take a sample, isolate a species, grow it as a pure culture on the appropriate media, and then examine it under a microscope. But not every microbe can be grown in the lab. DNA analysis expands what members of the microbial community we can see and study.

In prokaryotes, studying ribosomal DNA allows us to identify and compare relatedness of microbial species. The 16S gene, which makes up part of the 30S ribosomal subunit, is highly conserved from an evolutionary standpoint. (In other words, it has not changed much over time in a given species.) Looking at a microbe's 16S gene is therefore useful. Although complete genome sequences, not just 16S genes, are needed to identify a species, the more similar the 16S gene is to another known microbe's, the more closely related the two

species are. Automated systems using genetic analysis have been developed. These systems can rapidly recognize individual organisms from a mixed sample and are thus useful for identifying members of the intestinal microbiome, where a wide variety of bacteria coexist.

Genetic identification techniques have revolutionized the study of microbes. However, that doesn't mean Petri plates and microscopes are now obsolete. Genetic analysis isn't appropriate for all situations and won't tell us all the information we may want to know about a microbe in a sample. For example, genetic analysis may not detect species if they are low in number in the sample. Additionally, differences within a species, such as a new antibiotic-resistance gene, can be identified only by culturing. Growing bacteria is also essential to understanding their metabolism and their relationship to their host—both

crucial factors when it comes to studying microbiota. So in the end, we need both new and old techniques to properly study the microbiome.



Microbes grown from a 5-year-old's handprint.

DAFTAR PUSTAKA

1. Tortora GJ, Funke BR, Case CL, 2007, Microbiology an Introduction, 13th edition, Benjamin Cummings, An imprint of Addison Wesley, Longman Inc., USA

