Microbial groups

CE 421/521

Lecture September 14, 2006

Vaccari et al., Chapter 10

Microbes

- Microorganisms – broad category of organisms too small to be seen with the naked eye
- Integral part of every ecosystem
- Roughly 10^6 to 10^9 per gram of soil, biofilm or sludge sample

Microbial groups

- Prokaryotes
  - Bacteria (Including blue green algae)
  - Archae – (sometimes archaebacteria)
    - classified during the 1970's by Carl Woese and George Fox
    - Don't fit neatly into prokaryotic or eukaryotic class due to their difference in 16S rRNA - separate kingdom?
    - Includes methanogens and halophiles
- Viruses – Dimitri Ivanovsky (1893) filtered sap through ceramic filters designed to remove bacteria – still resulted in tobacco mosaic virus
- Eukaryotes

Classification of microorganisms

- Energy source:
  - Chemotrophs – energy from chemical substances
    - Organotrophs – energy from organic compounds
    - Lithotrophs – energy from an inorganic compound
  - Phototrophs – energy from sunlight

Classification of microorganisms

- Carbon source:
  - Heterotrophs – carbon from organic compounds
  - Autotrophs – carbon from inorganic compounds

  Can have mixed classifications:

  e.g. chemoorganoheterotroph (example E. Coli)

  chemolithoautotroph (example nitrobacter)

Classification of microorganisms

- Environmental preferences:
  - TEA (anaerobic, aerobic, anoxic)
  - Temperature
    - Psychrophiles
    - Mesophiles
    - Thermophiles
  - pH
    - Neutrophiles (5-9)
    - Acidophiles (< 5)
    - Alkaliphiles (> 9)
- Extremophiles – can grow at extreme temperatures or osmotic pressures (e.g., halophiles)

Microbial Taxonomy

- Morphology: form and visible structure
  - Biocehmcial activities
  - Phenotype – representing observable characteristics
- Genotype
  - Characterized by DNA or RNA
  - Phylogeny – based on genetic similarities

Taxonomy - What is a prokaryotic species?

- Difficulty in that genetic exchange occurs between species not necessarily closely related
  - Strain
    - have a recent parent cell
    - Share genetic properties with minor exceptions
  - Species
    - Share at least 70% of DNA homology – similarity in DNA sequence
    - Or have rRNA similarity of 97% or greater
  - Genus
    - Share at least 20% of their DNA homology
    - Or have rRNA similarity of 93-95%

Nomencalture

1. E_____ (e.g., *aquaticus, marina, coli*)
2. H_____ (e.g., *bovus, avium*)
3. Environmental c_____ (e.g., *thermophilus, halophilus*)
4. S_____ (e.g., *ovalis, longum, spaericus*)
5. C_____ (e.g., *aureus, niger*)
6. S_____ (e.g., *denitrificans, avium*)
7. P_____ (e.g., *methanobacterium, cerevisiae*)
8. D_____ (e.g., *typhi, botulinum, pneumoniae*)
9. P_____ (e.g., *winogradskii, burkholderia*)

Prokaryotes - shape

- cocci (spherical, e.g., *Streptococcus*)
- bacilli (rod shapes, e.g., *Bacillus subtilis*)
- spirilla (spiral, e.g., *Spirillum volutans*)
- filamentous

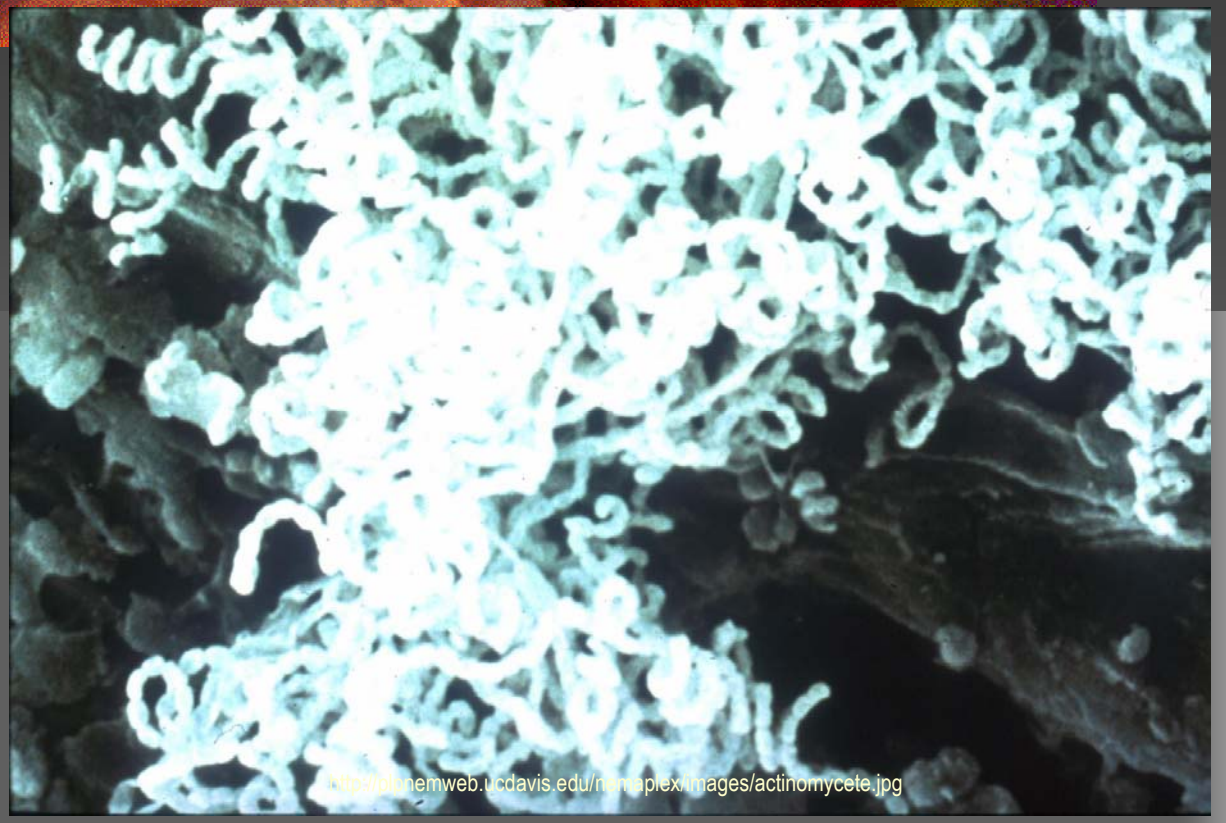
Prokaryotes - shape

- Unusual
  - s_____ bacteria - filamentous, surrounded by a sheath
  - s_____ bacteria - aerobic, gram negative, at end of stalk is a “holdfast” allows it to attach to surfaces
  - b_____ bacteria, multiply by budding, bud grows flagellum, settles on new surface and buds again
  - g_____ bacteria, filamentous, gram-negative, “glide” along solids surfaces, *Beggiatoa* and *Thiothrix*: oxidize H_2S to S^0

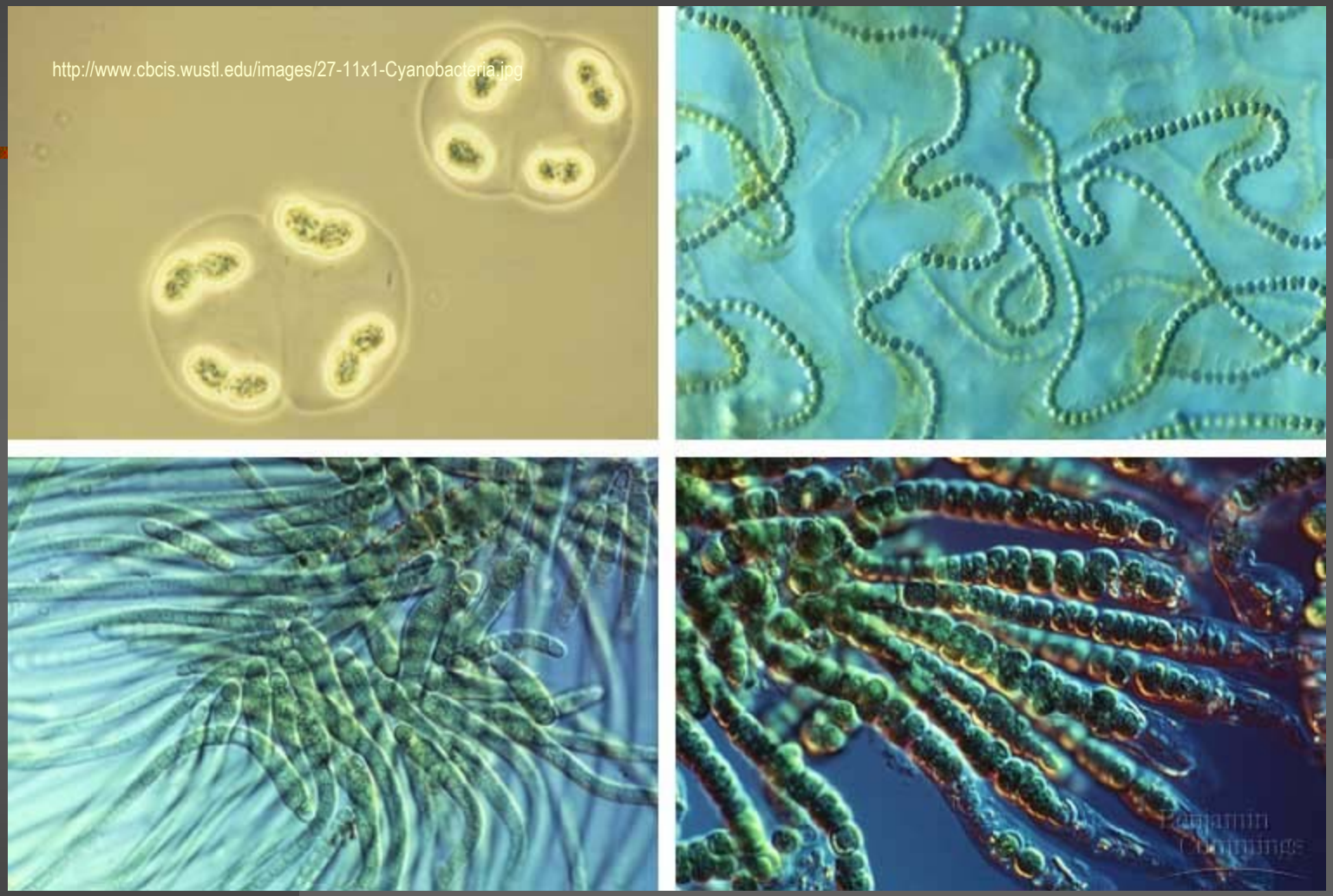
Prokaryotes - shape

- *Bdellovibrio* - s_____ (0.2-0.3μ) flagellated bacteria that prey on gram-negative bacteria
- Actinomycetes- gram-positive, f_____, have branching filaments similar to fungi - *Streptomyces* and *Nocardia*
- Cyanobacteria - b_____-g_____ algae, procaryotes, contain chlorophyl a, have characteristic blue-green color, contain gas vacuoles that enable them to float to maximize photosynthesis, responsible for algal blooms, some are toxic

Bdellovibrio



Actinomycetes



Cyanobacteria

Fungi

- e_____, produce long filaments called hyphae containing c_____
- heterotrophs, use o_____ compounds for carbon and energy
- found during n_____ limitations, low D.O., low pH conditions
- important in the cycling of organics – degradation of plant polymers cellulose and lignin
- primarily aerobic (except for fermentative yeast)

Fungal cell wall

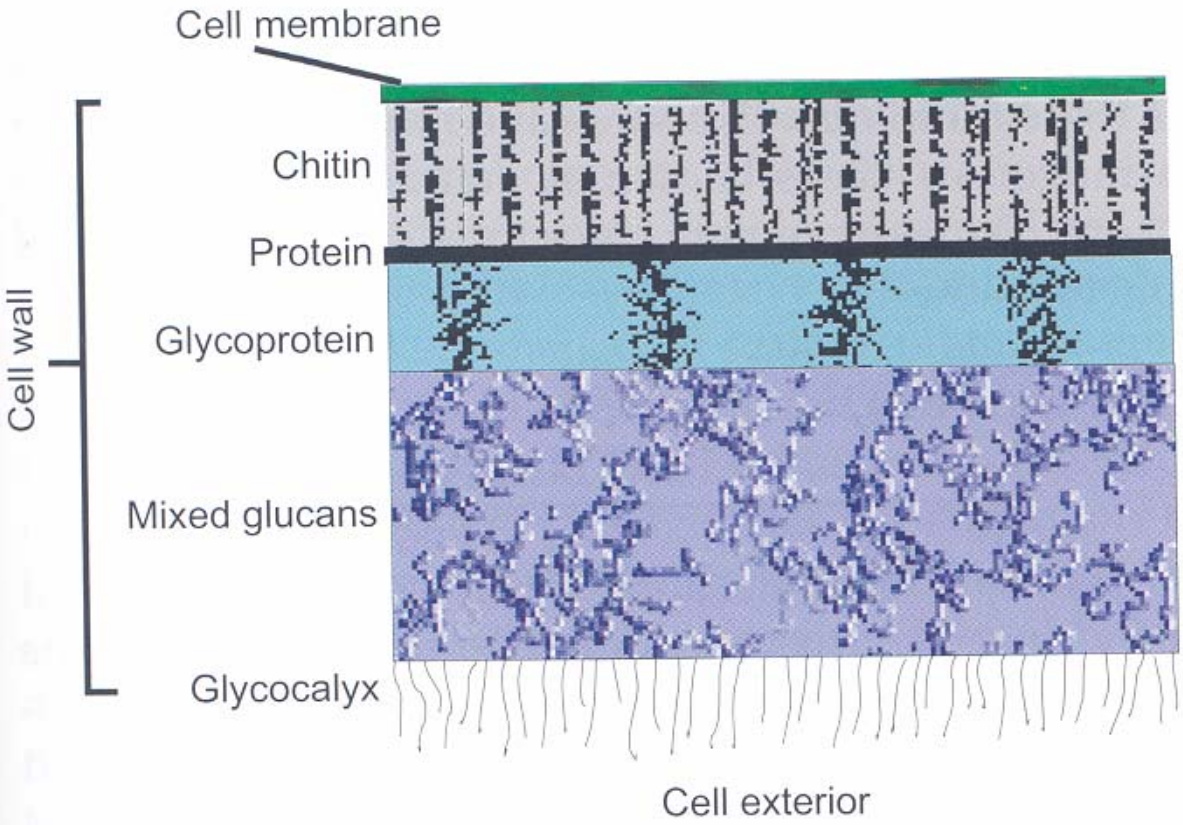


FIGURE 2.17 Structure of a fungal cell wall.

Algae

- most are u_____, floating, phytoplankton
- some are f_____
- most are p_____
- all contain chl_____ a, some b and c
- found in o_____ ponds, polishing ponds, aerobic lagoons

Algal Cell

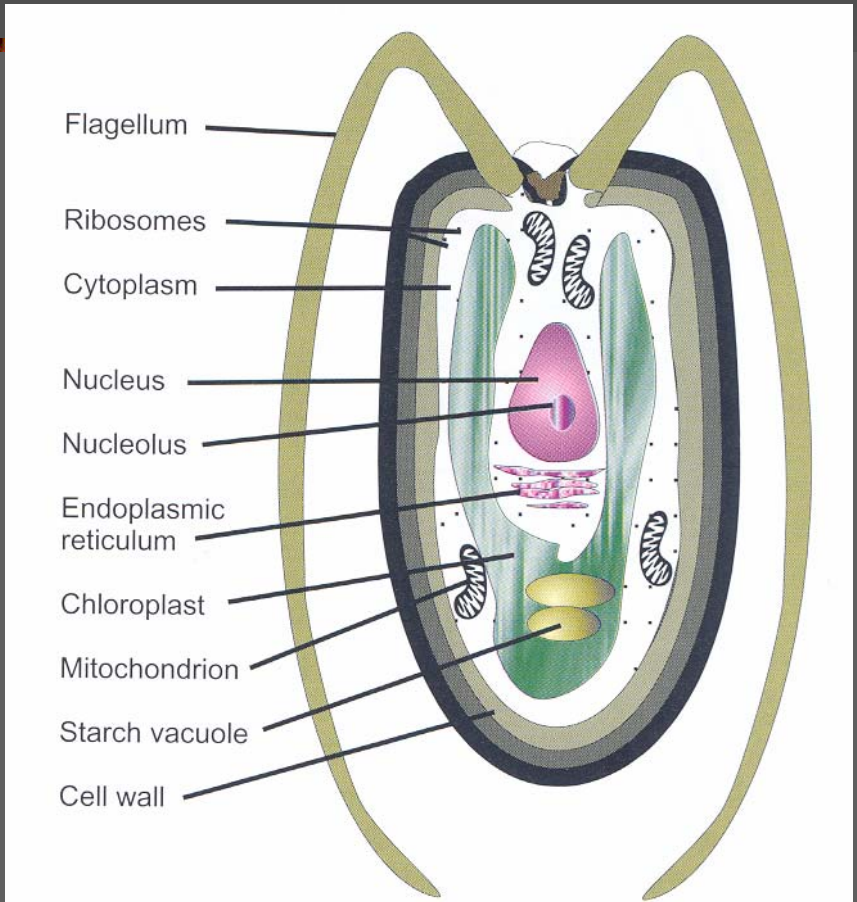
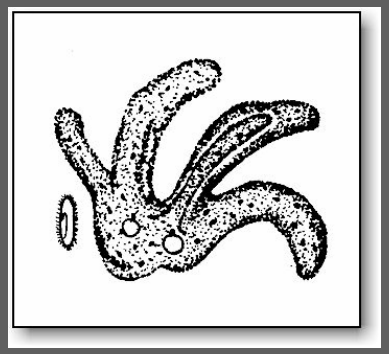
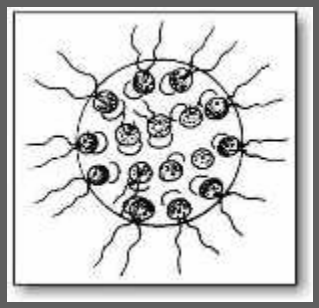


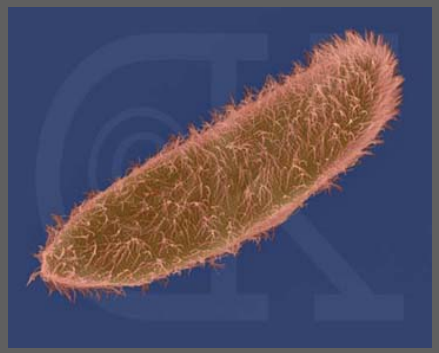
FIGURE 2.18 Structure of a typical algal cell.

Protozoa

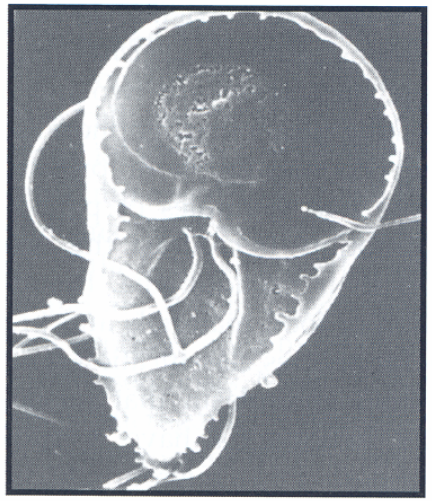
- Unicellular
- Heterotrophs
- Classification
  - sarcodina (amoebae)
  - mastigophora (flagellates)
  - ciliophora (ciliates)
  - sporozoa







A



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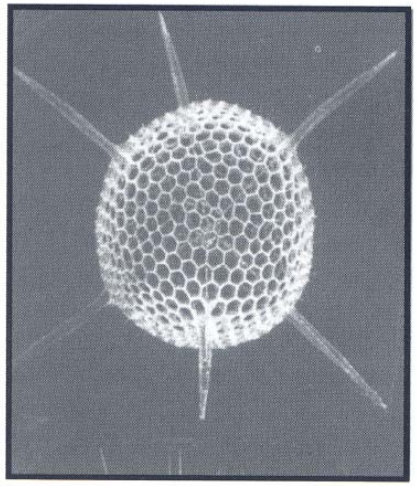

C

FIGURE 2.19 Basic morphology of protozoa. (A) scanning electron micrograph of a flagellar protozoa, *Giardia*; (B) scanning electron micrograph of testate cilia, *Heliosoma*; (C) electron micrograph of ciliated *Didinium*. Part A reprinted with permission from Cox (1993). Parts B and C reprinted with permission from Sleigh (1989).

Viruses

- small c_____ particles (not procaryotes or eucaryotes) are they alive?
- replication occurs in h_____
- Structure
  - c_____ of nucleic acid (could be double or single stranded, DNA or RNA) surrounded by protein coat (capsid)
  - main shapes
    - h_____
    - p_____
    - c_____

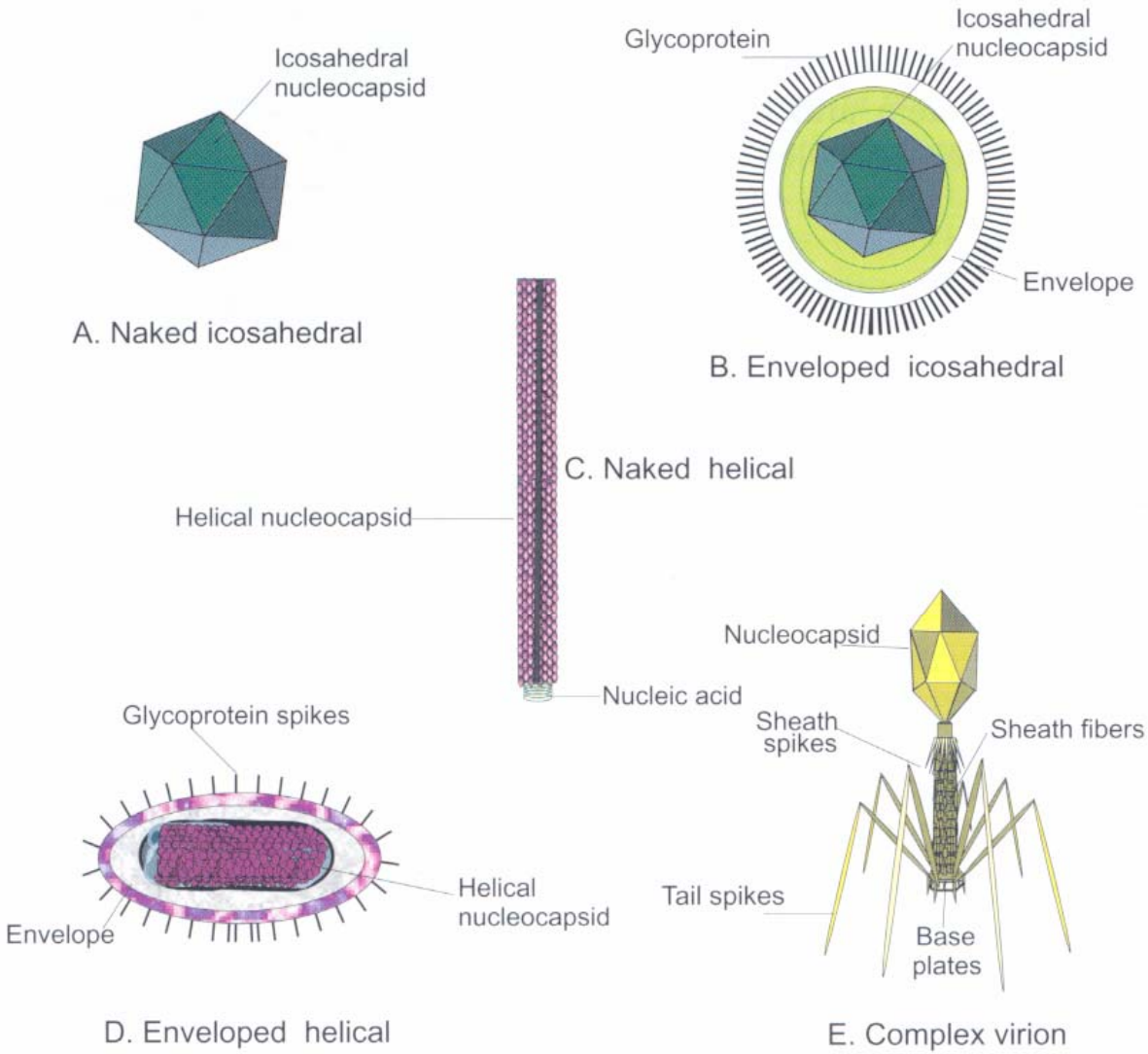

FIGURE 2.4 Simple forms of viruses and their components. The naked icosahedral viruses (A) resemble small crystals: the enveloped icosahedral viruses (B) are made up of icosahedral nucleocapsids surrounded by the envelope: naked helical viruses (C) resemble rods with a fine regular helical pattern in their surface: enveloped helical viruses (D) are helical nucleocapsids surrounded by the envelope: and complex viruses (E) are mixtures of helical and icosahedral and other structural shapes.

Virus Replication

1. Ad_____ - virus adsorbs to specific receptors, receptors can be polysaccharides, proteins, or lipoproteins
2. En_____ - various particle or nucleic acid material enters cell
3. Ec_____ - capsid is stripped away, releasing genetic material
4. Mu_____ - viral nucleic acids are replicated using machinery of host cell
5. Ma_____ - protein coat is synthesized and combined with nucleic acid to form nucleocapsid
6. Re_____ of mature virions - host cell ruptures release active viruses

Virus Detection and Enumeration

- animal i_____ - newborn mice injected with inoculum and observed for signs of disease
- t_____ cultures - viruses quantified by measuring effect on host cell lines forming a monolayer on glass or plastic assay bottles, effect is measure by
- p_____ assay - virus is placed on surface of host cell monolayer, virus replication leads to localized area of cell destruction called plaques
- s_____ dilution endpoint - virus suspension is diluted serially and the highest dilution (smallest amount of virus) that causes a cytopathic effect in 50% of samples is reported as the tissue culture infectious dose (TCID50)
- most p_____ number - serial dilutions placed in tubes or microwells with host cells, positive tubes are recorded and MPN value computed from standardized MPN table.

MPN

- Uses serial dilutions and statistical probabilities for the most likely number of organisms giving a positive response

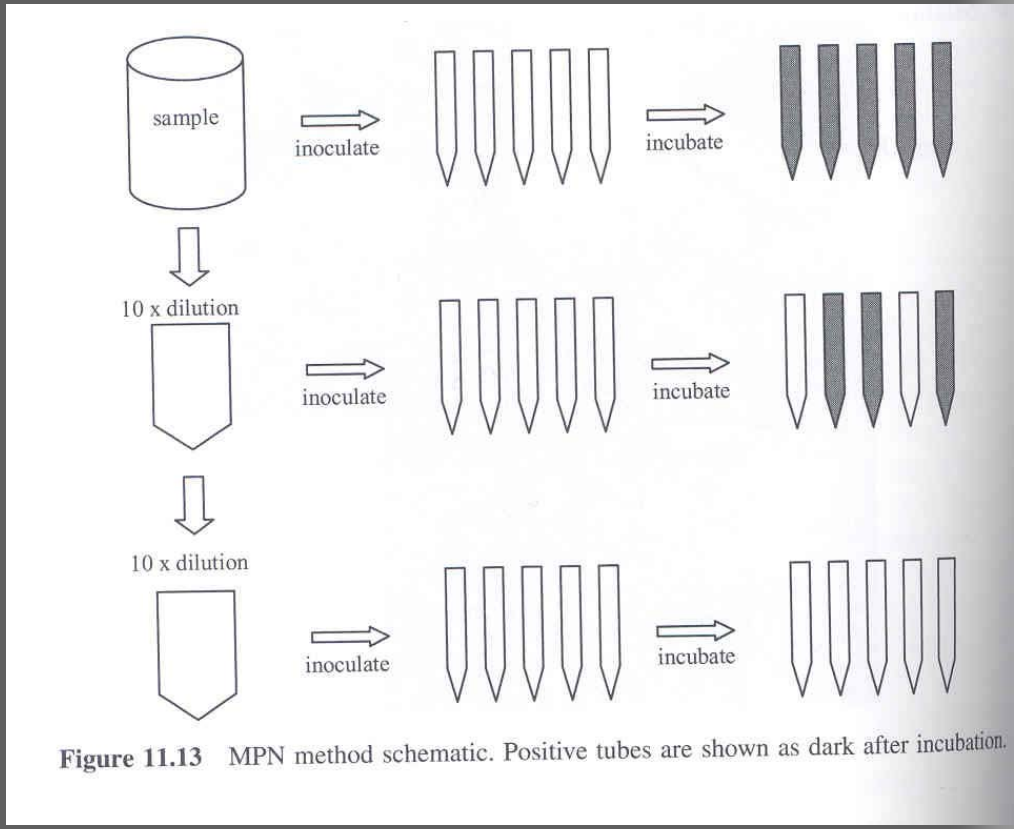

Figure 11.13 MPN method schematic. Positive tubes are shown as dark after incubation.

Example: Take 1 mL of sample and add to 1 L of water then perform the following serial dilutions:10 mL, 1 mL, and 0.1 mL and incubate with substrate. If we get 5 positive tubes in the first dilution, 4 positive tubes in the second dilution, and 1 in the last dilution, what is the MPN of the sample?

Solution: from the following table we can see that the as diluted MPN is 170. Since we had a 1000 fold dilution to start with, the resulting MPN is 170,000 organisms per 100 mL

Number of positive tubes				Number of positive tubes				Number of positive tubes				Number of positive tubes				Number of positive tubes				Number of positive tubes			
10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN
0	3	0	5.6	1	3	0	8.3	2	3	0	12	3	3	0	17	4	3	0	27	5	3	0	79
0	3	1	7.4	1	3	1	10	2	3	1	14	3	3	1	21	4	3	1	33	5	3	1	110
0	3	2	9.3	1	3	2	13	2	3	2	17	3	3	2	24	4	3	2	39	5	3	2	140
0	3	3	11	1	3	3	15	2	3	3	20	3	3	3	28	4	3	3	45	5	3	3	180
0	3	4	13	1	3	4	17	2	3	4	22	3	3	4	31	4	3	4	52	5	3	4	210
0	3	5	15	1	3	5	19	2	3	5	25	3	3	5	35	4	3	5	59	5	3	5	250
0	4	0	7.5	1	4	0	11	2	4	0	15	3	4	0	21	4	4	0	34	5	4	0	130
0	4	1	9.4	1	4	1	13	2	4	1	17	3	4	1	24	4	4	1	40	5	4	1	170
0	4	2	11	1	4	2	15	2	4	2	20	3	4	2	28	4	4	2	47	5	4	2	220
0	4	3	13	1	4	3	17	2	4	3	23	3	4	3	32	4	4	3	54	5	4	3	280
0	4	4	15	1	4	4	19	2	4	4	25	3	4	4	36	4	4	4	62	5	4	4	350
0	4	5	17	1	4	5	22	2	4	5	28	3	4	5	40	4	4	5	69	5	4	5	430
0	5	0	9.4	1	5	0	13	2	5	0	17	3	5	0	25	4	5	0	41	5	5	0	240
0	5	1	11	1	5	1	15	2	5	1	20	3	5	1	29	4	5	1	48	5	5	1	350
0	5	2	13	1	5	2	17	2	5	2	23	3	5	2	32	4	5	2	56	5	5	2	540
0	5	3	15	1	5	3	19	2	5	3	26	3	5	3	37	4	5	3	64	5	5	3	920
0	5	4	17	1	5	4	22	2	5	4	29	3	5	4	41	4	5	4	72	5	5	4	1600
0	5	5	19	1	5	5	24	2	5	5	32	3	5	5	45	4	5	5	81				