Chapter 4B: Methods of Microbial Identification

Chapter Reading – pp. 118-121, 244-245, 250-251
Biochemical Testing

In addition to morphological (i.e., appearance under the microscope) and differential staining characteristics, microorganisms can also be identified by their biochemical “signatures”:

- the nutrient requirements and metabolic “by-products” of a given type of microorganism

![Fermentation test](image)

- test media typically change color when particular components are metabolized and associated by-products are released
methods of rapid identification

- commercial devices to carry out multiple biochemical tests simultaneously to rapidly produce a panel of results
Use of Dichotomous Keys

A series of “yes/no” questions to ID organism.

1a. Gram-positive cells
1b. Gram-negative cells
2a. Rod-shaped cells
2b. Non-rod-shaped cells
3a. Can tolerate oxygen
3b. Cannot tolerate oxygen
4a. Ferments lactose
4b. Cannot ferment lactose
5a. Can use citric acid as a sole carbon source
5b. Cannot use citric acid alone
6a. Produces hydrogen sulfide gas
6b. Does not produce hydrogen sulfide gas
7a. Produces acetoin
7b. Does not produce acetoin
8a. Produces gas from glucose
8b. Does not produce gas from glucose

(a)

(b)
Serological Testing

Using specific antibodies to reveal the presence of antigens unique to specific microorganisms.

- antibodies are produced in animals in response to anything “foreign”
- injection of animal with a foreign protein will result in antibodies to it
- antibodies present in the animal serum can then be used in various tests such as the agglutination test

Agglutination test

(a) Negative result
(b) Positive result
Phage Typing

Bacteriophages (viruses that infect bacteria) have very specific hosts and can be used to ID bacteria:

- grow a “lawn” of bacteria to be tested on agar plate
- “dot” different test phage samples on surface
- after ~24 hr, plaques appear where bacteria have been infected & killed
- profile of phage sensitivity can reveal ID of bacteria
DNA Base Composition

Members of the same genera or species have nearly identical DNA sequences, and hence the same proportions of G/C base pairs & A/T base pairs:

• because they base pair, \( G = C \) and \( A = T \)

• \( G/C + A/T = 100\% \) (e.g., if \( G/C = 40\% \) then \( A/T = 60\% \))

Determining the G/C content of the DNA from a test organism and comparing to known values is a quick way to eliminate possible identities:

• if \( \%G/C \) is different, cannot be a match!

• if \( \%G/C \) is same, might be a match but additional testing is necessary to confirm
The Use of DNA Hybridization

With enough heat, DNA strands will separate. Cooling allows complementary strands to base pair.

- this technique is used in a variety of ways to see if DNA from two different sources are similar
- usually the DNA from one source is immobilized, the other is labeled to allow detection
Fluorescent in situ hybridization:

1) label DNA “probe” (fr. species of interest) w/fluorescent tag
2) chemically treat cells to allow DNA to enter, hybridize
3) wash & view with fluorescence microscopy
**cells w/DNA complementary to probe will fluoresce!**
PCR Amplification

Polymerase Chain Reaction can be used to selectively amplify DNA sequences of interest (if there to begin with):

- requires DNA to be tested, sequence specific primers, heat-stable DNA polymerase, free nucleotides (dNTPs)

- essentially DNA replication in vitro

- alternating cycles of heating & cooling allow exponential amplification of target DNA

(b) Each PCR cycle doubles the number of copies of the DNA
Prokaryotic ribosomes contain 3 different rRNA mol.:  
- large subunit contains 23S (2900 nt) & 5S (120 nt) rRNA  
- small subunit contains 16S (1500 nt)  

16S rRNA sequence is typically used for ribotyping:  
- sequence is highly conserved (varies little)  
- degree of difference reflects “evolutionary distance”  
  **primary method for classifying prokaryotic species**
Key Terms for Chapter 4B

- dichotomous key, serological, phage typing
- DNA hybridization: FISH, PCR
- ribotyping

Relevant Chapter Questions:

none