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The Identification of Unknown Bacteria

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THE IDENTIFICATION OF UNKNOWN BACTERIA

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A major activity of most college level introductory microbiology courses is the identification of one or more unknown bacterial species. The ways suggested in many lab manuals for identifying bacteria are often unorganized and difficult to follow. In addition, the huge volume of information often required to make a diagnosis may overwhelm beginning microbiology students who are largely incapable of weighing the importance of many of the tests in trying to decide how to best proceed with the identification. Microbiology manuals too often overlook the fact that identification is an *orderly process* of moving from general characteristics to more specific differences between closely related organisms.

In introducing bacterial identification to the beginning student:

1. The number of possible species must be limited to a manageable size.
2. Students should have at least some familiarity with these taxa from working with them in previous laboratory exercises.
3. The number of criteria needed to identify a bacterial species should be minimized.
4. The identification process should require some familiarity with *Bergey's Manual of Determinative Bacteriology.*
5. The process of taxonomic identification must proceed in an orderly, predictable sequence of choices beginning with higher taxonomic levels and proceeding to the level of the species.

The following procedure is designed with the above suggestions in mind.

Suggested Procedure for Identification of Unknown Bacteria:

1. The identification process should begin with a thorough investigation of the colonial characteristics, microscopic morphology, motility, oxygen requirements and staining characteristics of the unknown bacteria. If a mixture of bacteria is used, the student must first isolate each species. The resulting information can be used to determine to which "Part" in *Bergey's Manual* the species belongs.
2. The student then performs a battery of primary physiological tests in conjunction with controls. The instructor should emphasize that all tests may not be needed to identify a particular species of bacteria. The results from the controls will determine the reliability of each test.

3. Once this testing is completed, the student begins to identify the genus of his unknown. Twenty-one genera covering eight parts or sections in *Bergey's Manual* have been selected as the core of this identification procedure. Most of these genera are commonly used in microbiology labs and should, therefore, be somewhat familiar to the students. All are easily obtainable from most biological supply companies. The accompanying dichotomous key will allow the student to identify the genus of bacteria with a minimum of difficulty. The key uses only those characteristics which provide consistently dependable results. Whenever possible those biochemical tests which are difficult to perform, or are often unreliable, are omitted.
4. Once the student has identified the bacterial genus, he then verifies it by comparing his test results with those listed in the accompanying table. Table 1 presents information from *Bergey's Manual* in a simple, standardized and easily useable form. The data given in Table 1 may differ from that presented in the manual since test data for rare species have been excluded. However, symbols used in this table are identical with those used in *Bergey's Manual.*
5. Only after the student has made an accurate generic identification is he ready to proceed to the species level. To identify the species, the student must refer to characteristics presented in *Bergey's Manual.* In our lab we usually designate a group of possible species within each genus. This is done to eliminate rare or unusual species for which we lack the proper media for proper physiological identification and to standardize the level of complexity for all students.

We find the use of this identification procedure extremely effective in our microbiology courses and hope that others will find it to be an equally effective teaching tool.

Reference

Buchanan, R. E. and N. E. Gibbons (Eds.). 1974. *Bergey's Manual of Determinative Bacteriology.* Williams and Wilkins Co., Baltimore.

A KEY TO COMMON GENERA OF BACTERIA

1. a. Gram negative 2
   b. Gram positive 7
2. a. Curved rods, motile 3
   b. Not as above 4
3. a. Helical cells, microaerophilic to anaerobic ..... *Spirillum*
   b. Straight or slightly curved rods, aerobic ..... *Pseudomonas*
4. a. Rods 5
   b. Cocci, often paired ..... *Neisseria*

A KEY TO COMMON GENERA OF BACTERIA (CONTINUED)

5. a. Aerobic or microaerophilic, ovoid to almost coccoid pleomorphic rods, often Gram variable 6
   b. Faculative anaerobes 10
6. a. Large ovoid cells, 2 mm or more in diameter, occur singly, in pairs, or irregular clumps ..... *Azotobacter*
   b. Single straight or curved rods, 0.5-4 μm ..... *Pseudomonas*
7. a. Cocci 17
   b. Rods 8
8. a. Straight rods 9
   b. Irregular rods or tending to form filaments 20
9. a. Cells rod shaped, endospores formed, often motile 21
   b. Straight or curved rods, occuring singly or in chains, no endospores formed, nonmotile ..... *Lactobacillus*
10. a. Lactose fermented rapidly 11
    b. Lactose not fermented or fermented slowly 14
11. a. Hydrogen sulfide produced ..... *Citrobacter*
    b. Hydrogen sulfide not produced 12
12. a. Indole produced ..... *Escherichia*
    b. Indole not produced 13
13. a. Motile ..... *Enterobacter*
    b. Nonmotile ..... *Klebsiella*
14. a. Hydrogen sulfide produced 15
    b. Hydrogen sulfide not produced 16
15. a. Urea fermented rapidly, phenylalanine deaminase present .... *Proteus*
    b. Urea not fermented or fermented slowly, phenylalanine deaminase absent ..... *Salmonella*
16. a. Citrate used as sole carbon source ..... *Serratia*
    b. Citrate not used as sole carbon source ..... *Shigella*
17. a. Aerobes, glucose not fermented (may be oxidized) 18
    b. Faculative anaerobes, glucose fermented 19
18. a. Spherical cells, occurring in regular tetrads or packets, endospores formed ..... *Sporosarcina*
    b. Spherical cells, occurring singly, in pairs or irregular clusters, no endospores formed ..... *Micrococcus*
19. a. Grow in presence of 15% NaCl; catalase positive ..... *Staphylococcus*
    b. No growth in 15% NaCl; catalase negative ..... *Streptococcus*
20. a. Acid fast negative rods showing pleomorphism, pallisades and metachromatic granules ..... *Corynebacterium*
    b. Acid fast positive rods or coccoid elements sometime forming filaments ..... *Mycobacterium*
21. a. Aerobes or faculative anaerobes ..... *Bacillus*
    b. Strict anaerobes ..... *Clostridium*

Table 1
Summary characteristics of common bacterial genera.

Part (Bergey's Manual)	6	7		8								10	14			15			16	17	
General Characteristics	SPIRAL & CURVED	GRAM NEGATIVE AEROBIC RODS AND COCCI		GRAM NEGATIVE FACULTATIVE ANAEROBIC RODS								GRAM NEG COCCI & COCCOBACILLI	GRAM POSITIVE COCCI			ENDOSPORE FORMING RODS & COCCI			GRAM POS NONSPORE-FORMING RODS	ACTINOMYCETES AND RELATED ORGANISMS	
Genus	SPIRILLUM	AZOTOBACTER	PSEUDOMONAS	ESCHERICHIA	CITROBACTER	SALMONELLA	SHIGELLA	KLEBSIELLA	ENTEROBACTER	SERRATIA	PROTEUS	NEISSERIA	MICROCOCCUS	STAPHYLOCOCCUS	STREPTOCOCCUS	SPOROSARCINA	BACILLUS	CLOSTRIDIUM	LACTOBACILLUS	CORYNEBACTERIUM	MYCOBACTERIUM
Growth Characteristic			best growth at 25-30° C							pink pigment on NA at room te		some produce yellow/green pigment	yellow or red pigment								
Microscopic Morphology	rigid helical cells	large ovoid cell single, pairs, clumps	straight or curved rods	short rod single or paired	short rod single or paired	short rods	short rods	short rod single, paired or chains	short rods	short rods	short rod single, paired or chains	diplococci with joined sides flattened	cocci, single, paired, tetrads	staphylococci	paired cocci or chains	cocci in tetrads or packets	long rods	rod or club shaped	short rod or coccobacilli	rods, often club shaped, pleomorphic,	straight or curved rods and coccoid elements
Oxygen Requirements	microaerophil or anaerobes	aerobes or microaerophils	aerobes	facultative anaerobes	facultative anaerobes	facultative anaerobes	facultative anaerobes	facultative anaerobes	facultative anaerobes	facultative anaerobes	facultative anaerobes	aerobes or facultative anaerobe	aerobes	facultative anaerobes	facultative anaerobes	aerobes	aerobes or facultative anaerobes	anaerobes	anaerobes	facultative anaerobes or anaerobes	aerobes
Size	.25-1.7 μm	≥ 2 μm	.5-1 by 1.5-4 μm	1-3 by .4-.7 μm	1-3 by .4-.7 μm			.3-1.5 by .6-6 μm			.6 by 3 μm	.6-1 μm	.5-3.5 μm	.5-1.5 μm	< 2 μm	.3-2.2 by 1.2-7 μm	.3-2.2 by 1.3-7 μm				.2-.6 by 1-10 μm
Motility	bipolar, peritrich	+ or —	polar	+ or —	peritrich	peritrich	—	—	peritrich	peritrich	peritrich	—	—	—	—	+ or —	lateral	peritrich	—	—	—
Gram Stain	—	v	—	—	—	—	—	—	—	—	—	—	+	+	+	+	v	+	+	+	v
Acid Fast Stain	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Spore Stain	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—			—	—	+	D
Oxidase	+		+	—	—	—	—	—	—	—	—	+							—		
Nitrate Reduced	D		D	+	+	+	+	+	+	+	+		D	+		+	D	D	—	D	D
Growth on 10-15%NaCl	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	D				
Glucose	—			+	+	+	—	d	+	d	D	+	D	+	+	—	+	D	+	D	
Lactose	—			+	+	D	D	D	+	—	D		—	D	D		D	D	+	D	
Maltose	—			+	+	+		+	+	+	D	D		D	+		D	D	+	D	
Mannitol	—	D		+	+	+	D	+	+	+	D			D	D		D	D			D
Sucrose	—			d	d	—	D	+	+	+	D	D		+	+		+	D			
Starch		D	—	—					D		—	D	—	—	D	—	D	D			
Citrate	—			—	+	+	—	+	+	+	D						D				D
Methyl Red				+	+	+	+	—	—	—	+		—								
Voges Proskauer				—	—	—	—	+	+	+	d										
Gelatin Hydrolysis	—		D	—	—	D	—	—	(+)	+	D		D	+	—	—	D	D	—		
H_2S Produced	+			—	D	+	—	—	—	—	D	D						D			
Indole	—		—	+	—	—	D	—	—	—	+		—					D			
Urease	D			—	(+)	—	—	—	—	—	+										D
Phenylalanine Dase	—			—	—	—	—	—	—	—	+										

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