



دانشگاه اصفهان

دانشکده علوم و فناوری های زیستی، گروه سلولی و مولکولی و میکروبیولوژی ،
آزمایشگاه میکروبیولوژی

آزمایشگاه باکتری شناسایی ۲

رنگ آمیزی گرم و آشنایی با خصوصیات میکروسکوپی و ماکروسکوپی باکتری های
جنس سودوموناس

PSEUDOMONAS

- A large group of aerobic, non sporing gram negative bacteria motile by polar flagella
- Found in nature water, soil, other moist environments
- Some of them are pathogenic to plants
- Creation of new genera such as Burkholderia, Stenotrophomonas

- Large group of aerobic
non sporing
gram negative
motile by polar flagella



- ubiquitous
- opportunistic infections
- newer genera-Burkholderia



Stenotrophomonas

Pseudomonas aeruginosa

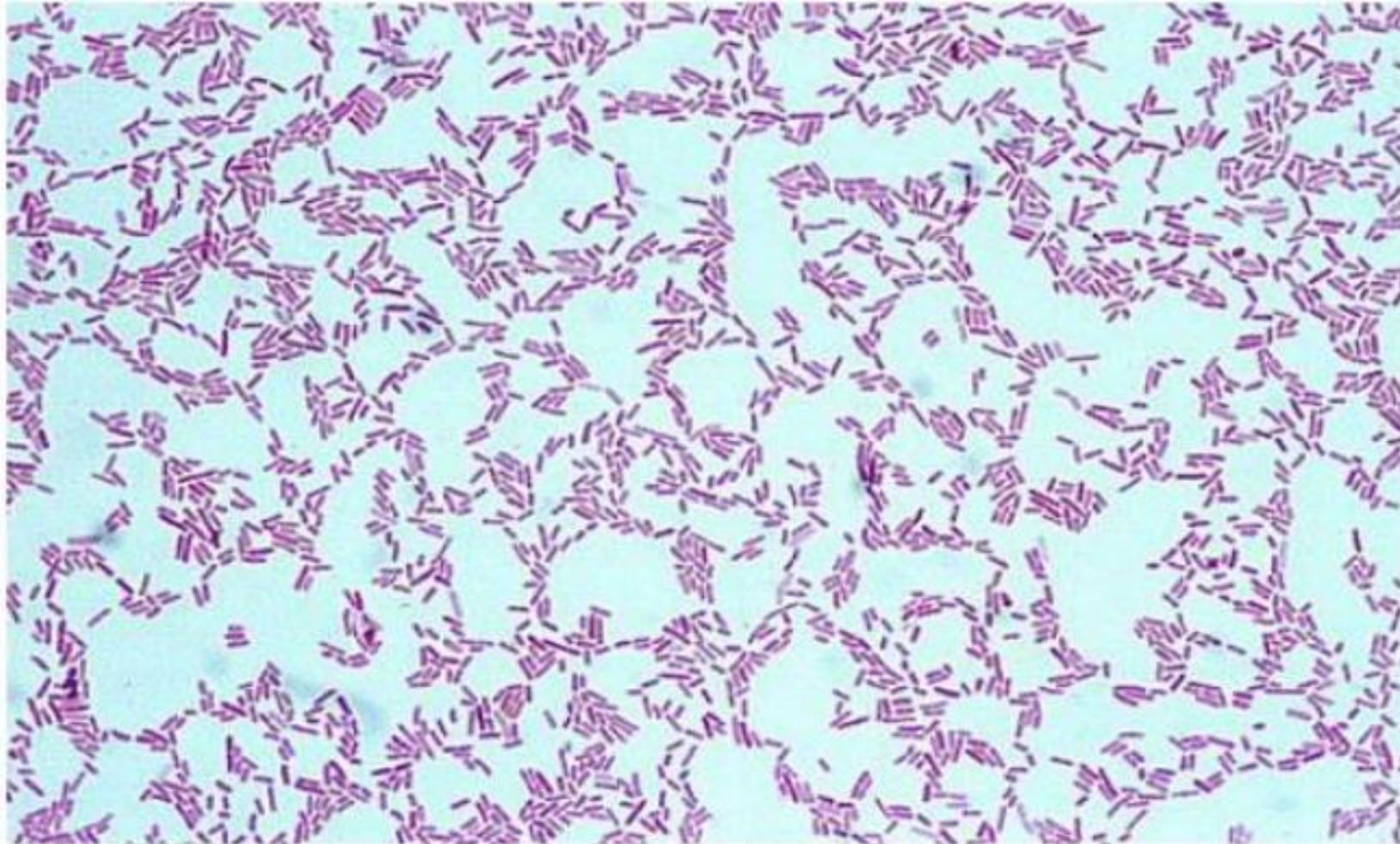
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تهیه کننده: سهیلا عباسی

MORPHOLOGY

- They are slender gram negative bacillus, 1.5 – 3 microbes x 0.5 microns
- Monoflagellar ?
- Non capsulated but many strains have mucoid slime layer
- Isolates from Cystic fibrosis patients have abundance of extracellular polysaccharides composed of alginate polymers
- Escape the defence mechanisms by loose capsule in which micro colonies of bacillus are enmeshed and protected from host defences.



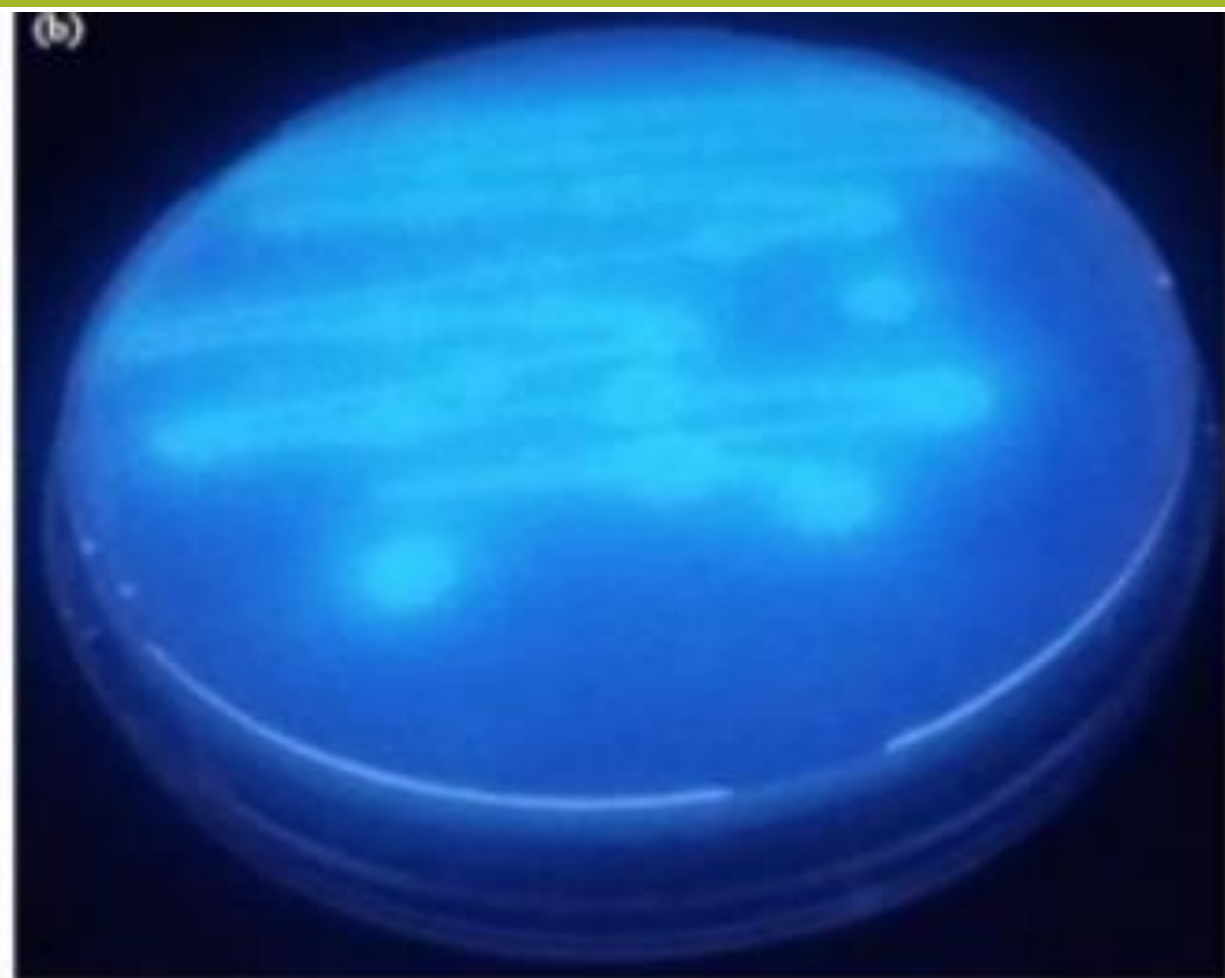
Pseudomonas aeruginosa is a Gram negative, non-sporing motile rod

CULTURAL CHARACTERS

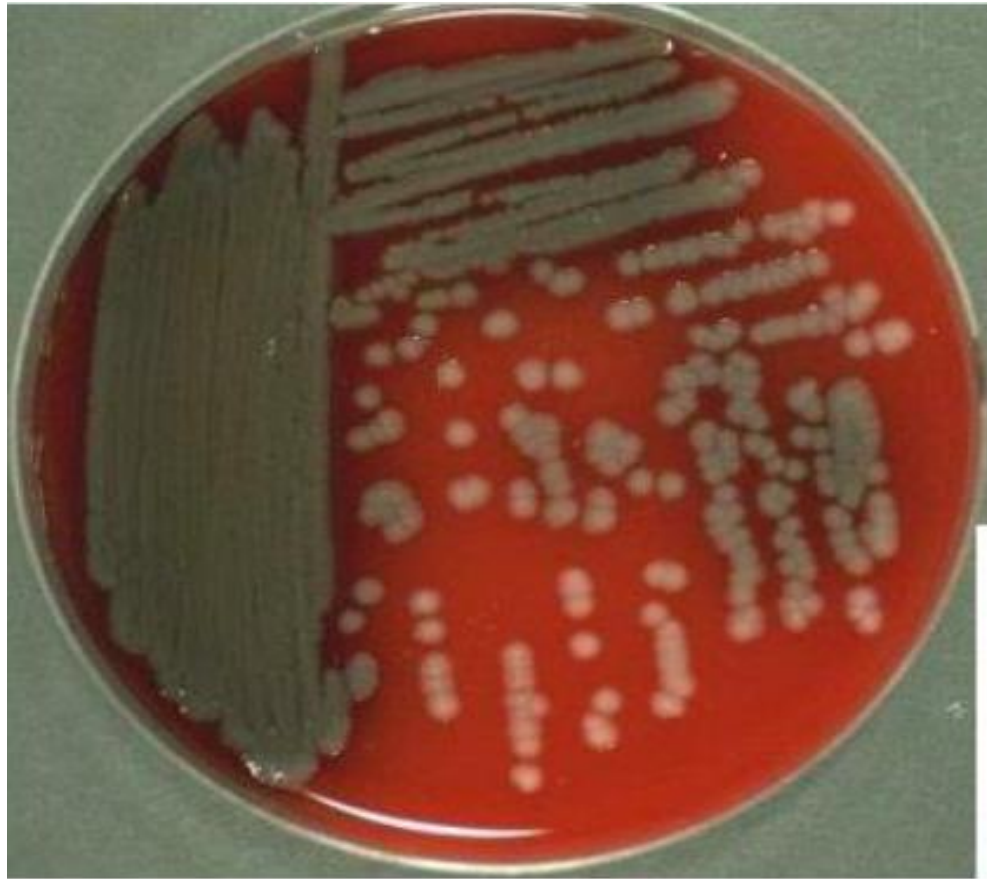
- Obligate aerobe, but grow anaerobically if nitrate is available
- Growth occurs at wide range of temperatures 6-42 c the optimum being 37 c
- Growth on ordinary media producing large opaque irregular colonies with distinctive musty mawkish or earthy smell.
- Iridescent patches with metallic sheen are seen in cultures on nutrient agar.
- In broth forms dense turbidity with surface pellicle.



On Nutrient agar *P. aeruginosa* can be recognized by the pigments, it produces a blue-green pigment (pyocyanin).



Pseudomonas aeruginosa on *Pseudomonas cetrimide* agar, (a) *P. aeruginosa* under light lamp showing green pyocyanin pigment and (b) *P. aeruginosa* give fluorescence under UV lamp



P. aeruginosa produces

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large, flat, spreading colonies which are often haemolytic and usually pigment-producing.

The pigments diffuse into the medium giving it a dark **greenish-blue** colour





ASM MicrobeLibrary.org © Buxton

P. aeruginosa produces pale coloured colonies on MacConkey agar

Species

- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia*
- *Burkholderia cepacia*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*

Characteristics of Pseudomonas aeruginosa

- **Motile** (by single or multiple polar flagella)
gram-negative rods
- Obligate (strict) **aerobes** (most strains)
- **Oxidase** (usually) and **catalase positive**
- Nonfermentative chemoheterotrophic
respiratory metabolism
- **Minimal nutritional reqts.;** **Many organic compounds used** as C and N sources, but only a few carbohydrates by oxidative metabolism
 - Glucose used oxidatively
 - **Lactose negative** on MacConkey's agar

PIGMENT PRODUCTION

- Some strains produce diffusible pigments:
 - Pyocyanin (blue); fluorescein (yellow); pyorubin (red)
- *P. aeruginosa* produces characteristic grape-like odor and blue-green pus & colonies
- Broad antibiotic resistance

Pyocyanin



Pyoverdinin



Pigment production

■ *Pyocyanin*

- Bluish green phenazine pigment
- Soluble in chloroform and water
- Not produced by other species

■ *Pyoverdinin(flouorescin)*

- It is a greenish yellow pigment
- Insoluble in chloroform but soluble in water
- Produced by many other species

Cont..

- *Blood agar*

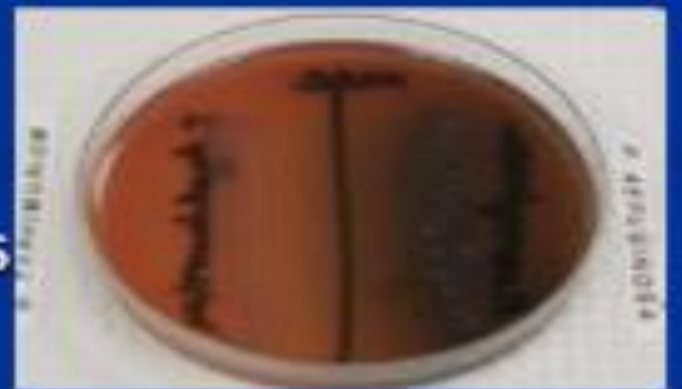
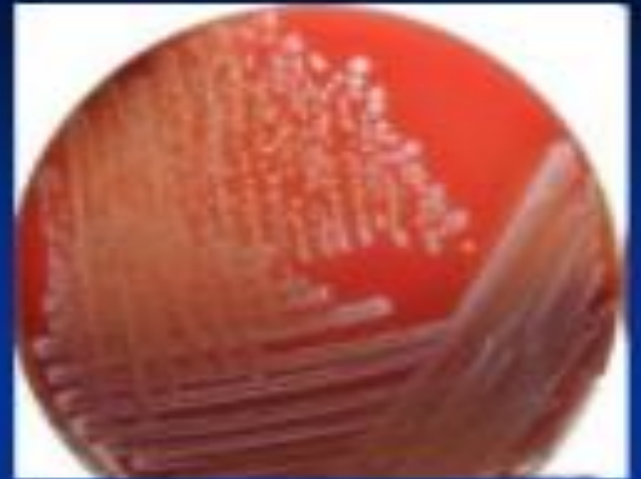
- Similar to nutrient agar
- Many are haemolytic

- *Mac conkey agar*

- Colourless, non lactose fermenters

- *Cetrímide agar*

- selective media



Cont..

■ *Pyorubin*

- Reddish brown pigment
- Insoluble in chloroform but soluble in water

■ *pyomelanin*

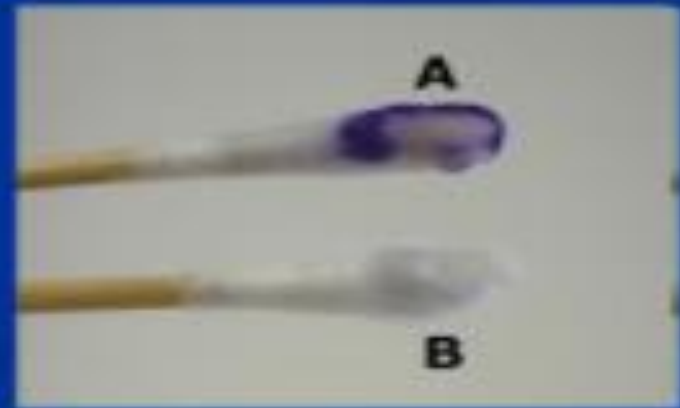
- Brown to black pigment
- Production is uncommon

BIOCHEMICAL REACTIONS

- Oxidative and Non fermentative
- Glucose is utilized oxidatively
- Indole, MR and VP and H₂ S tests are negative
- **Catalase, Oxidase, and Arginine tests are positive**

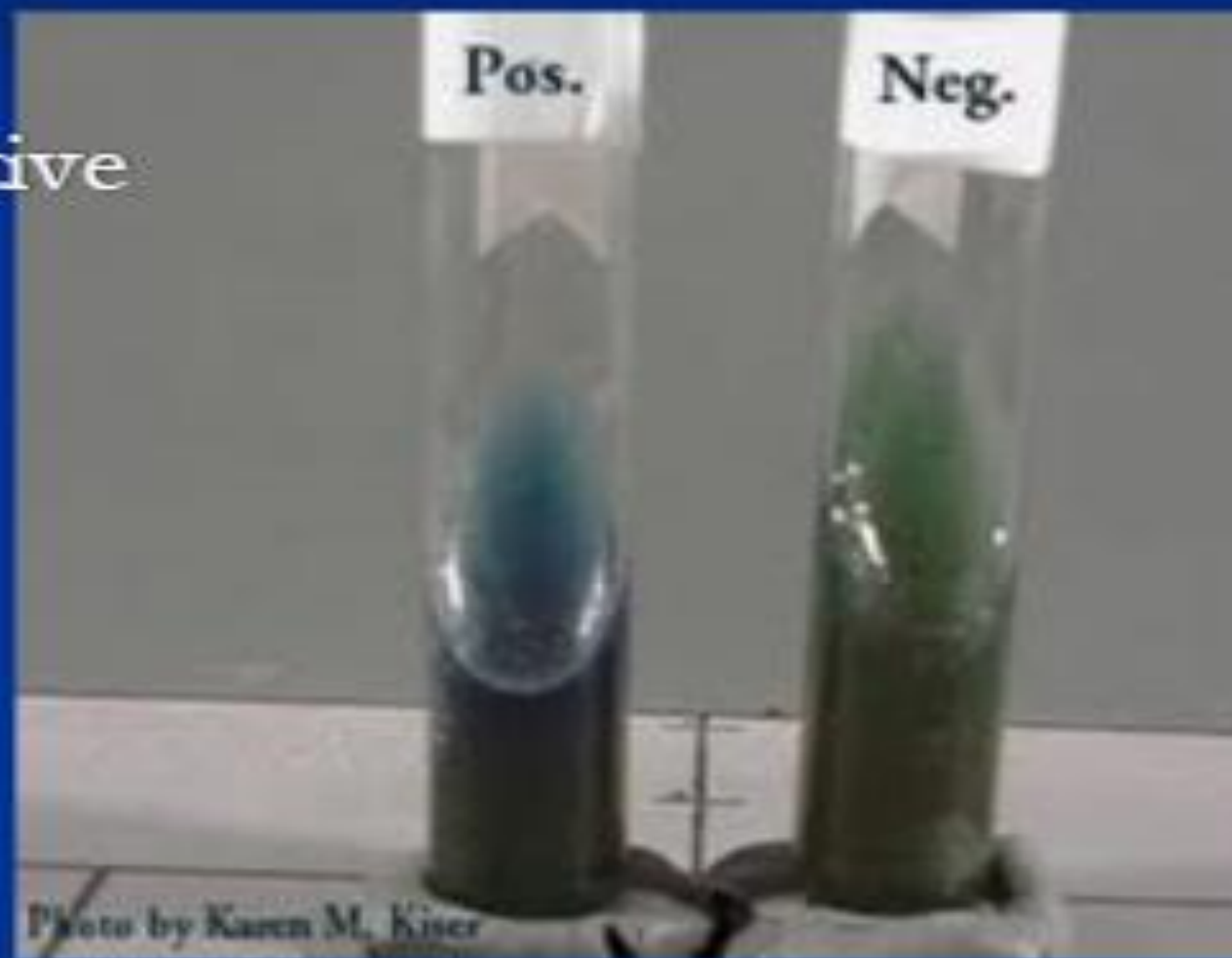
Biochemical reactions

- O/F test-oxidative
- Catalase-positive
- Oxidase-positive
- Nitrate reduction-positive



Cont..

- Indole test-negative
- Methyl red test-negative
- Vp test-negative
- Citrate test-positive
- Urease test-negative

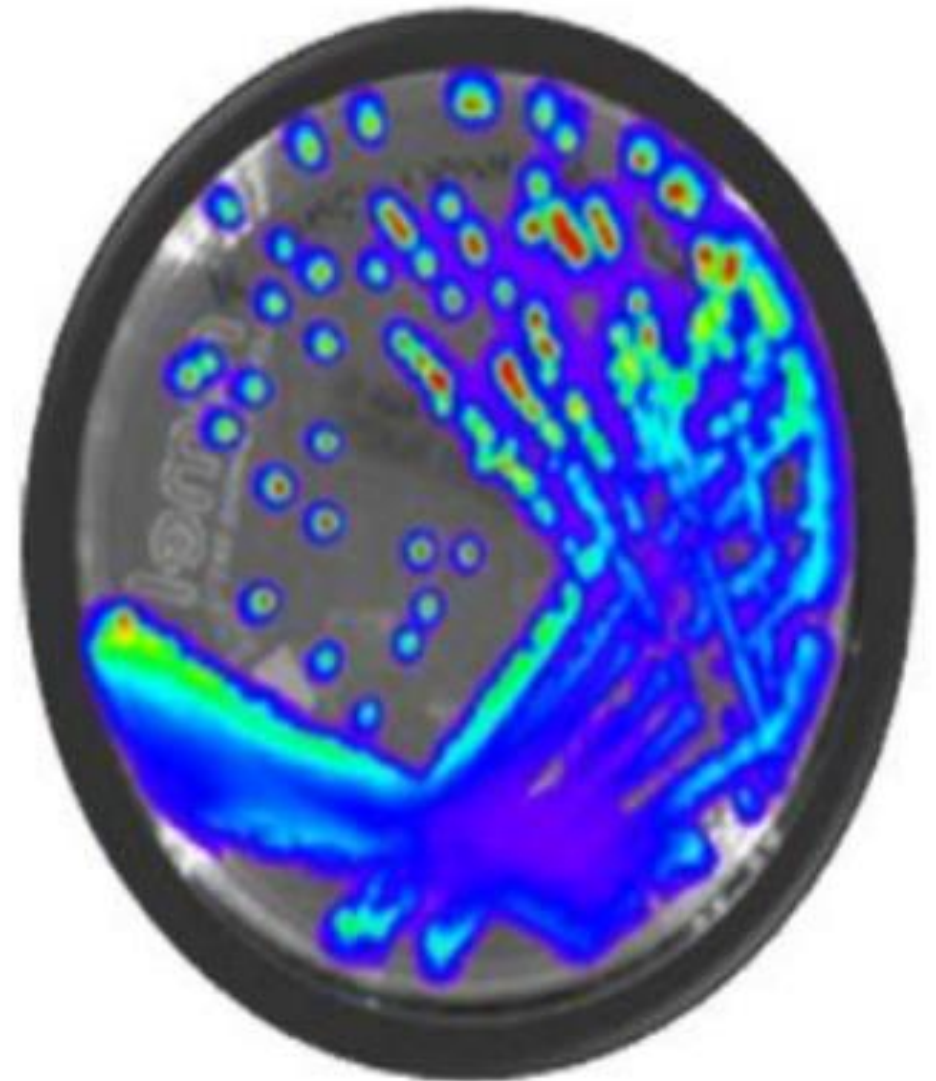


Cont...

- *Sugar fermentation tests*
 - Glucose-only acid
 - Lactose-negative
 - Sucrose-negative
 - Mannitol-negative

pseudomonas

- Toxic extracellular products in culture filtrates
- Exotoxin A and S
- Exotoxin A acts as NADase resembling Diphtheria toxin
- Proteases, elastase, hemolysins and enterotoxin
- Slime layer and Biofilms



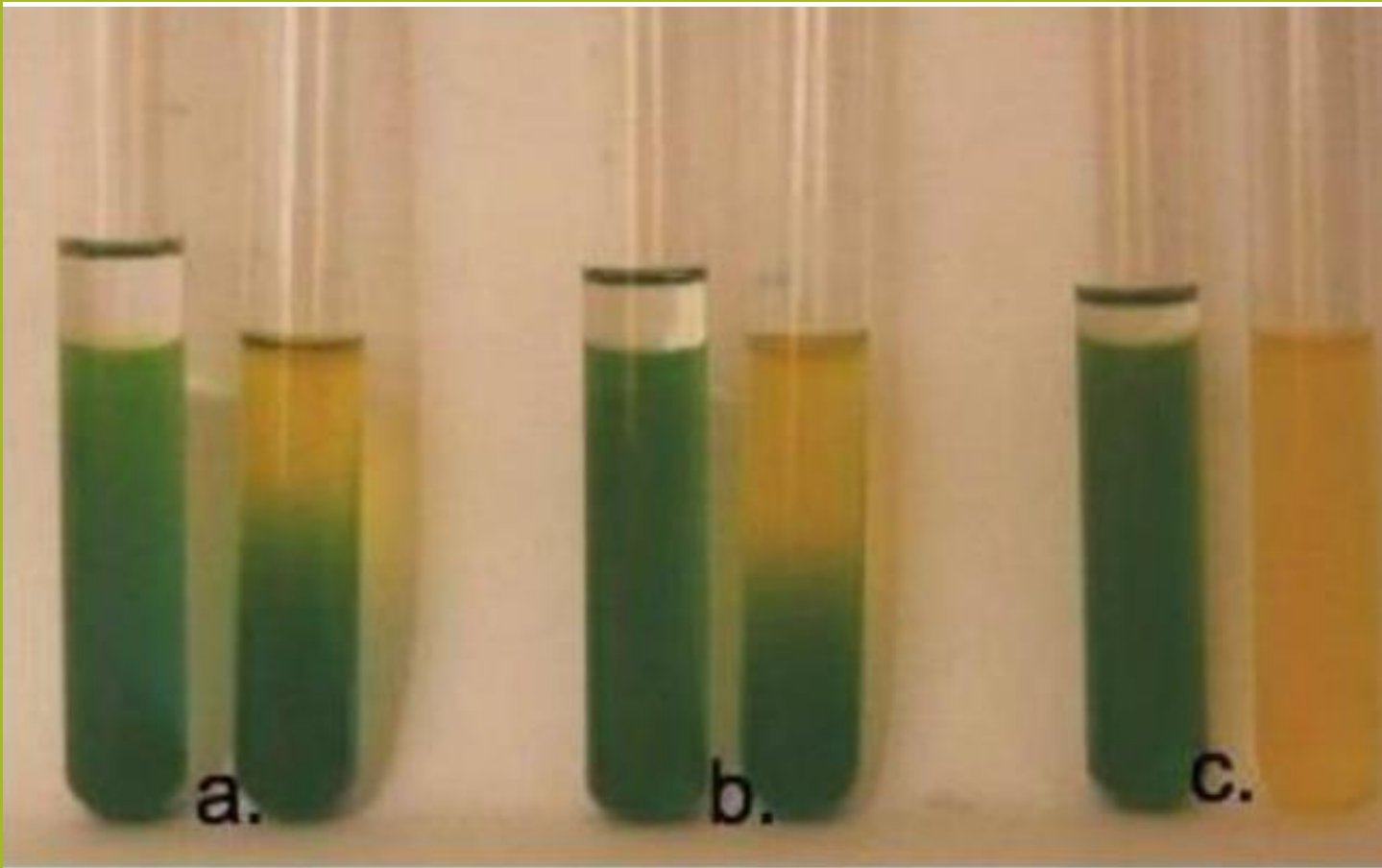
O-F TEST

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The oxidative-fermentative test determines if certain gram-negative rods metabolize glucose by **fermentation** or aerobic respiration (**oxidation**). During the **anaerobic** process of glucose **fermentation**, the high concentration of acid produced will turn the bromthymol blue indicator in OF media from green to yellow in the presence or absence of oxygen

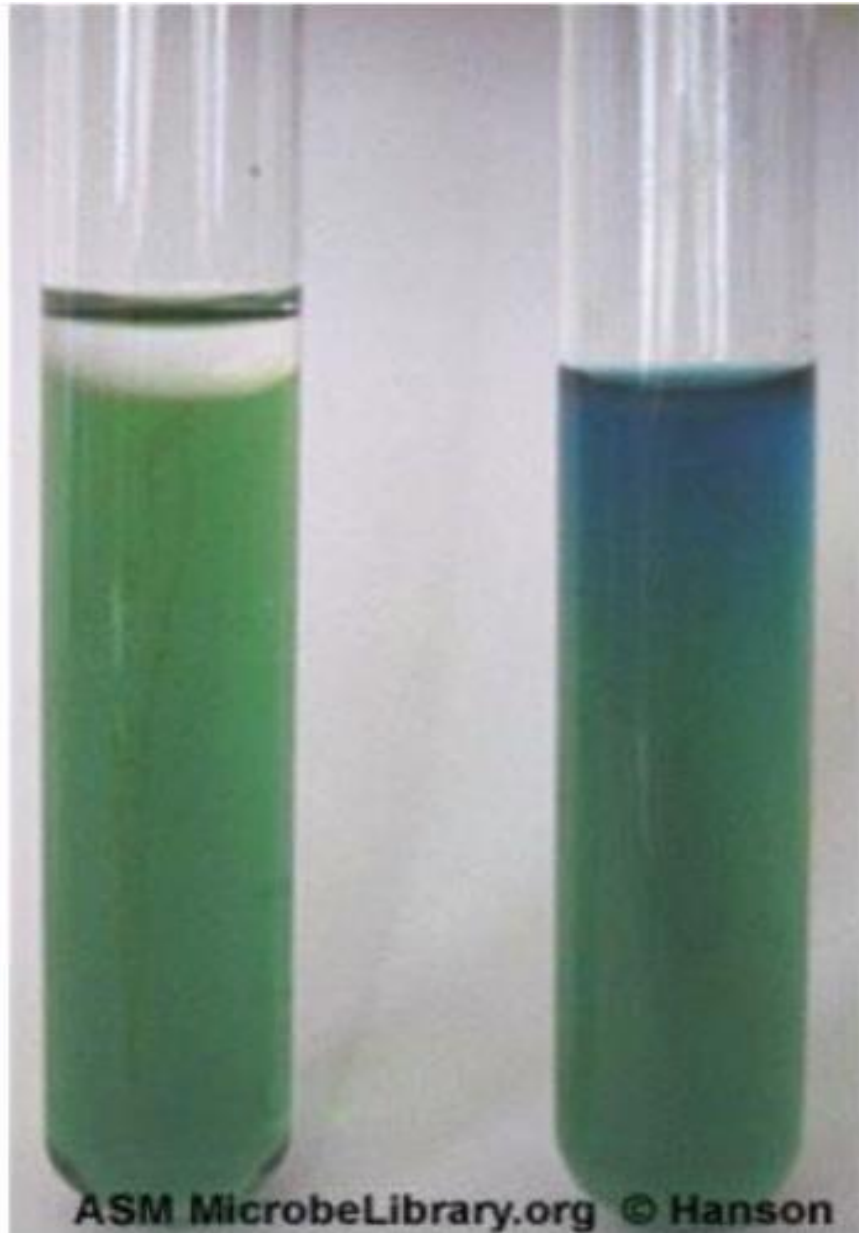


Acid production in
both the open and
oil-covered tubes
indicates a
fermentative result
(e.g. *Escherichia*
coli)



- (a) *P. aeruginosa* incubated for 24 hours.
- (b) *P. aeruginosa* incubated for 48 hours.
- (c) *P. aeruginosa* incubated for 5 days.

Acid production in the open tube and not the oil-covered tube indicates an **oxidative** result.



No color change in the oil-covered tube and color change to alkaline in the open tube indicates a negative result. *A. faecalis* cannot use glucose **fermentatively** or **oxidatively**. The blue at the top of the open tube is due to amine production resulting from the metabolism of protein in the media.

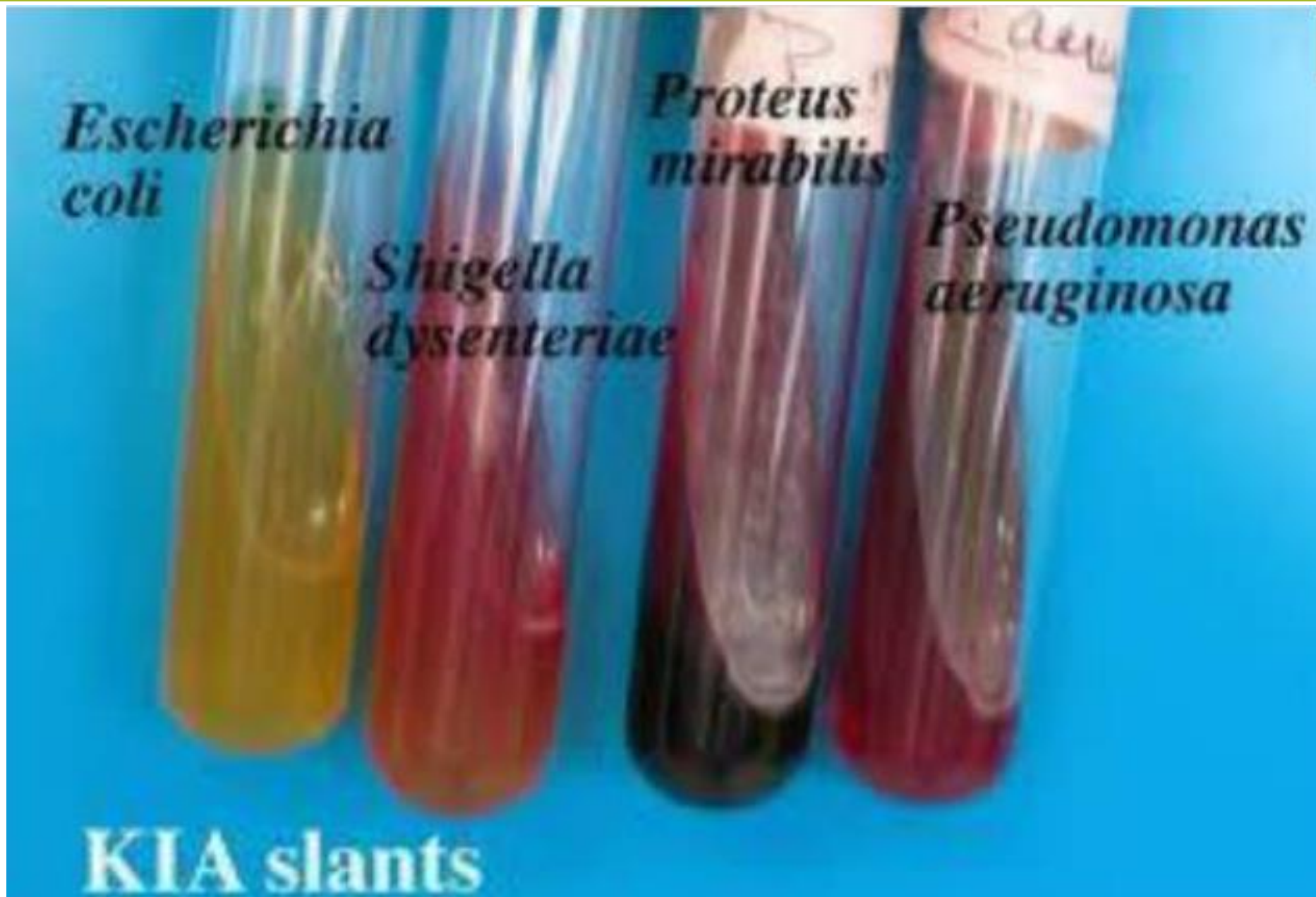


Table 7.5. Second-stage table for *Achromobacter*, *Alcaligenes*, *Bordetella*, *Shewanella* and the alkali-producing pseudomonads

	1	2	3	4	5	6	7	8	9	10	11
Brown pigment	-	-	-	-	-	d	-	-	-	-	-
Orange pigment	-	-	-	+	-	-	-	-	-	-	-
Oxidase	+	+	+	+	+	-	+	+	+	+	+
Nitrate reduced	+	+ ^a	-	+	+	-	+	+	-	+	d
Simmons' citrate	+	d	d	-	+	-	+	+	-	d	+
Christensen's citrate	+	+	+	d	+	d	+	+	-	d	+
Urease	-	-	-	d	+	+	d	-	-	d	d
Gelatinase production	-	-	-	+	-	-	-	-	+	-	-
Growth in KCN medium	d	+	d	-	+	-	-	-	d	-	d
H ₂ S from TSI	-	-	-	+	-	-	-	-	-	-	-
Ornithine decarboxylase	-	-	-	d	-	-	-	-	-	-	-
Casein hydrolysis	-	-	-	+	-	-	-	-	-	-	-
Deoxyribonuclease production	-	-	-	+	-	-	-	-	-	-	-
Carbohydrates [in ammonium salt medium], acid from:											
glucose	+	-	-	d	-	-	-	-	-	d	-
arabinose	-	-	-	d	-	-	-	-	-	-	-
ethanol	d	d	+	-	-	-	-	-	d	+	-
fructose	d	-	-	-	-	-	+	-	-	d	-
glycerol	-	-	-	-	-	-	+	-	-	d	d
maltose	-	-	-	d	-	-	-	-	-	-	-
mannitol	-	-	-	-	-	-	+	-	-	-	-
sucrose	-	-	-	d	-	-	-	-	-	-	-
xylose	+	-	-	-	-	-	-	-	-	-	-
Tween 20 hydrolysis	-	-	-	+	-	-	+	+	+	d	+
Tween 80 hydrolysis	-	-	-	+	-	-	+	+	-	-	+
Tyrosine hydrolysis	+	+	+	d	+	-	+	+	d	+	+
Tyrosine, brown pigment production from	-	-	-	d	-	+	-	d	+	d	-
Growth on b-hydroxybutyrate	+	+	+	+	+	-	+	d	+	+	+
PHBA* accumulation in cells	+	d	d	-	d	-	+	-	+	d	+
Growth on Cetrinide Agar	+	+	+	-	-	-	d	d	-	d	-
Growth at 42 °C	d	d	d	d	d	-	-	+	-	+	-
Lecithinase production	-	-	-	+	-	-	-	d	-	-	-

1 *Achromobacter xylosoxidans*;
Alcaligenes xylosoxidans subsp. *xylosoxidans*
2 *Alcaligenes denitrificans*;
Alcaligenes xylosoxidans subsp. *denitrificans*
3 *Alcaligenes faecalis*; '*A. odorans*'
4 *Shewanella putrefaciens*;
Alteromonas putrefaciens; '*Pseudomonas putrefaciens*'
5 *Bordetella bronchiseptica*;
'*Alcaligenes bronchisepticus*'

6 *Bordetella parapertussis*;
'*Haemophilus parapertussis*'; '*Alcaligenes parapertussis*'
7 *Pseudomonas acidovorans*
8 *Pseudomonas alcaligenes*
9 *Pseudomonas diminuta*
10 *Pseudomonas pseudoalcaligenes*
11 *Pseudomonas testosteroni*

RESISTANCE

- Killed at 55°C in 1 hour
- High resistance to chemical agents
- Resistance to quaternary ammonium compounds. Chloroxyleneol
- Resistant to Hexachlorophenes
- Grows also in antiseptic bottles
- Dettol as cetrimide as selective medium
- Sensitive to acids silver salts, beta glutaraldehyde

WHAT ANTIBIOTICS TO USE

- Aminoglycosides
- Gentamycin, Amikacin, Cephalosporins
- Cefotaxime. Ceftazidime. Ofloxacin,
- Piperacillin, ticarcillin
- Local application, colistin, polymyxin

PATHOGENICITY

- Blue pus
- Causing the nosocomial infection
- Suppurative otitis
- Localised and generalised infections
- Urinary tract infection after catheterization
- Iatrogenic meningitis
- Post tracheostomy pulmonary infections

Table 7.1. First-stage table for Gram-negative bacteria

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Shape	R	S	S	S	S/R	R	R	R	R	R	R	R	R	R	R	R/S	R	H/C	H/C	H	R	R	NT
Motility	-	-	-	-	-	-	-	-	+	+	-	-	+	+	D	-	+	+	+	+	+	+	NT
Growth in air	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Growth anaerobically	+	+	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	D	+	+	-	+
Catalase	d	D	+	+	+	+	+	+	+	+	+	+	+	+	+	D	-	D	+	-	-	+	?
Oxidase	-	?	+	+	-	+	+	+	+	+	+	+	+	+	-	D	+	+/w	+	-	-	D	?
Glucose (acid)	D	-	+	-	+	-	-	-	-	+	+	+	+	+	+	D	-	-	-	+	+	-	?
Carbohydrates [F/O/-]	F/-	-	O	-	O/-	F	-	-	-	O	O/-	F	F	F	F	NT	-	-	-	F	F	-	?

<i>Bacteroides</i>	+	+																					
<i>Fusobacterium</i> ^a	+	+																					
<i>Veillonella</i> ^b	+	+																					
<i>Neisseria</i>			+																				
<i>Branhamella</i>				+																			
<i>Acinetobacter</i>					+																		
<i>Kingella</i>						+																	
<i>Moraxella</i>							+																
<i>Brucella</i>								+															
<i>Bordetella pertussis</i>									+														
<i>Bordetella parapertussis</i>										+													
<i>Bordetella bronchiseptica</i>											+												
<i>Alcaligenes</i>												+											
<i>Shewanella</i>													+										
<i>Pseudomonas</i> (alkali-producers)														+									
<i>Achromobacter</i>															+								
<i>Agrobacterium</i> ^c																+							
<i>Janthinobacterium</i>																	+						
<i>Pseudomonas</i> (oxidizers) ^d																		+					
<i>Flavobacterium</i> ^e																			+				
<i>Actinobacillus</i>																				+			
<i>Pasteurella</i>																					+		
<i>Aeromonas salmonicida</i>																						+	
<i>Cardiobacterium</i>																							+
<i>Chromobacterium</i>																							
<i>Vibrio</i>																							
<i>Plesiomonas</i>																							
<i>Aeromonas</i>																							
Enterobacteria ^f																							
<i>Haemophilus</i>																							
<i>Gardnerella</i>																							
<i>Eikenella</i> ^g																							
<i>Campylobacter</i>																							
<i>Helicobacter</i>																							
<i>Arcobacter</i>																							
<i>Anaerobiospirillum</i>																							
<i>Streptobacillus</i> ^h																							
<i>Legionella</i>																							
<i>Mycoplasma</i>																							

* Generally poor growth in air; better growth in air + CO₂.

† No growth in air or anaerobically; growth in 3–10% O₂.

^a Also *Leptotrichia buccalis*

^b Also *Acidaminococcus* and *Megasphaera*

^c Also *Ochrobactrum*

^d Also *Weeksella*; do not attack carbohydrates

^e Also *Chryseomonas* and *Flavimonas*

^f Enterobacteria: *Buttiauxella*, *Cedecea*, *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Escherichia*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, *Tatumella*, *Yersinia* and others.

^g Also *Stenotrophomonas maltophilia*

□ Cultural characters of these organisms can be found in tables with the number indicated.

NT Not testable by usual methods. Fermentative (Sneath & Johnson, 1973).

⊕ Typical form
? Not known

Table 7.1. First-stage table for Gram-negative bacteria

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Shape	R	S	S	S	S/R	R	R	R	R	R	R	R	R	R	R	R/S	R	H/C	H/C	H	R	R	NT
Motility	-	-	-	-	-	-	-	-	+	+	-	-	-	+	D	-	-	+	+	+	-	+	NT
Growth in air	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-*	-†	+	-	+	+	+
Growth anaerobically	+	+	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	D	+	+	+	-	+
Catalase	d	D	+	+	+	-	+	+	+	+	+	+	-	+	+	D	-	D	+	-	-	+	?
Oxidase	-	?	+	+	-	+	+	-	+	+	+	+	+	+	-	D	+	+w	+	-	-	D	?
Glucose (acid)	D	-	+	-	+	-	-	-	-	+	+	+	+	+	+	D	-	-	-	+	+	-	?
Carbohydrates [F/O/-]	F/-	-	O	-	O/-	F	-	-	-	O	O/-	F	F	F	F	NT	-	-	-	F	F	-	?



Thank You