

Microbiology 20 Biochemical Unknown – Spring 2009 (due May 14th)

You should be prepared to turn in your notebook with your biochemical unknown identification completed after lab on Thursday May 14th. Out of a possible 70 points you will be graded as follows:

Successfully performing the *necessary* tests to identify your unknown (25 pts):

- Gram stain (verify with instructor)
 - morphology & arrangement
- capsule stain
- spore stain (only if *Gram-negative*)
- acid fast stain (only if *Gram-negative rod*)
- motility (via wet mount)
- colony characteristics on nutrient agar plate (see pg. 70 of lab manual)
 - color (observe color of colonies as well as any changes in color of agar)
 - diameter of typical *isolated* colony
 - overall appearance
 - margin
 - elevation
- color, growth characteristics on agar slant at 37° C and room temp (pg. 79)
- characteristics of broth culture (*before* mixing culture)
 - pellicle?
 - turbid?
 - flocculent?
 - sediment?
- *oxidation-fermentation/OF test
- *starch hydrolysis test (amylase)
- *PR-glucose, PR-lactose, PR-sucrose tests (fermentation)
- *methyl red/MR test (fermentation → mixed acids)
- *Voges-Proskauer/VP test (fermentation → acetoin)
- *citrate utilization test
- *gelatin hydrolysis test (gelatinase)
- *urea hydrolysis test (urease)
- *phenylalanine deaminase test
- *H₂S test
- *motility-indole-ornithine/MIO test
- *nitrate reduction test
- *oxidase test (cytochrome C oxidase)
- *catalase test

* Perform biochemical tests of unknown as part of Exercises 13 to 17.

Adequately recording the procedures and results of each test (25 pts):

- I (or anyone else) should be able to repeat your work using *only* your notebook
- your results should be recorded in color and/or sufficiently described and labeled
 - indicate the total magnification for all microscopic images
 - it should be clear on what date each test was done and analyzed
 - procedures already written out in your notebook can be referred to by page
- your notebook entries should be neat, well-organized, and easy to read
 - set aside a section of pages in your notebook to keep records of your unknown

Correctly interpreting your results and identifying your unknown (20 pts):

- correctly indicating what each result means (e.g., “pink” Gram stain = Gram-neg.)
- conclusion summarizing your results and the identity of your unknown
 - can be in chart, table or list form

****Verify identity of unknown using *Bergey’s Manual of Determinative Bacteriology*****

Possible Unknown Bacterial Species

Gram-negative

Aeromonas hydrophila
Alcaligenes faecalis
Citrobacter freundii
Enterobacter aerogenes
Enterobacter cloacae
Escherichia coli
Klebsiella pneumoniae
Proteus mirabilis
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorescens
Serratia marcescens
Shigella flexneri

Gram-positive

Bacillus cereus
Bacillus megaterium
Bacillus subtilis
Enterococcus faecalis
Lactococcus lactis
Micrococcus luteus
Micrococcus roseus
Mycobacterium phlei
Sporosarcina ureae
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pyogenes
Streptococcus salivarius

Gram-neg chart

	<i>A hydrophila</i>	<i>A faecalis</i>	<i>C freundii</i>	<i>E aerog</i>	<i>E cloacae</i>	<i>E coli</i>	<i>K pneum</i>	<i>P mirabilis</i>	<i>P vulgaris</i>	<i>P aeruginosa</i>	<i>P fluorescens</i>	<i>S marcescens</i>	<i>S flexneri</i>
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Arrangement	single	single	single	single	single	single	single	single	single	single	single	single	single
Pigments	-	[+]	-	-	-	-	-	-	-	fl. yellow agar	reddish orange	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	-	-	-	-	-	-	-	-	-	-	-
Indole	+	-	[-]	-	-	[+]	-	-	+	-	-	d	d
MR	[+]	-	+	-	[-]	+	+	+	+	-	[-]	+	+
VP	d	-	-	[+]	[+]	-	[-]	-	-	-	+	-	-
Citrate	d	[+]	+	+	+	-	d	d	d	+	+	+	-
Urease	-	-	d	-	d	-	+	+	+	[+]	d	[-]	-
PPA	d	-	-	-	-	-	+	+	+	-	-	-	-
Ornithine	-	-	d	+	+	d	-	+	-	-	+	+	-
Gelatinase	+	-	-	-	-	-	+	[+]	[+]	[+]	d	+	-
H ₂ S	d	-	+	-	-	-	-	+	+	-	-	-	-
Nitrate red.	+	-	+	+	+	+	+	+	+	denitrif.	denitrif. [-]	+	+
Starch	+	-	-	[-]	-	-	+	-	-	-	-	-	-
Glucose	+	-	+	+	+	+	+	+	+	-	d	+	+
Lactose	[-]	-	+	+	[+]	+	+	-	-	-	-	-	-
Sucrose	+	-	d	+	+	d	+	-	+	-	d	+	-
Gas	d	-	+	+	+	[+]	+	+	+	-	d	-	-
Mannitol	+	-	+	+	+	+	+	-	-	+	-	+	+
O/F	FA	OA	FA	FA	FA	FA	FA	FA	FA	OA	OA	FA	FA

FA facultative anaerobe

OA obligate aerobe

+ 90% or greater positive

- 90% or greater negative

d 26-75% of strains are positive

[+] 76-89% positive

[-] 76-89% negative

Gram-pos chart

	B cereus rod >3um pairs, chains	B megat rod pairs, chains	B subtilis rod <3um pairs, chains	E faecalis coccus pairs/chains	L lactis coccus pairs/chains	M luteus coccus tetrad/clusters	M roseus coccus tetrad/clusters	M phlei rod mycelium-like	S ureae coccus pairs, tetrads	S aureus coccus irreg clusters	S epiderm coccus irreg clusters	S pyogenes coccus pairs/chains	S salivarius coccus pairs/chains	Morphology Arrang.
Pigments	-	-	-	-	-	yellow	red	-	-	-	-	-	-	Pigments
Motility	+	+	+	-	-	-	-	-	+	-	-	-	-	Motility
Endospores	+	+	+	-	-	-	-	-	+	-	-	-	-	Endospores
Starch	[+]	+	+	+	-	-	[-]	-	-	[-]	[-]	d	d	Starch
Catalase	+	+	+	d	-	+	+	+	+	+	+	-	-	Catalase
Oxidase	[+]	-	-	-	-	+	d	-	+	-	-	-	-	Oxidase
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	Indole
VP	+	-	+	+	+	d	d	-	-	+	+	+	+	VP
Citrate	[+]	+	[+]	[-]	-	+	-	+	-	d	[-]	-	-	Citrate
Urease	[-]	[+]	-	-	-	d	-	+	+	+	+	-	[+]	Urease
Ornithine				-	-	-	-	-	-	-	-	-	-	Ornithine
PPA	-	-	-	-	-	-	-	-	d	-	-	-	-	PPA
Gelatinase	+	+	+	d	-	[+]	[-]	-	-	+	[+]	-	-	Gelatinase
Nitrate red.	+	-	+	-	-	[-]	[+]	+	+	+	+	-	-	Nitrate red.
Glucose	+	+	+	+	+	[-]	+	-	-	+	+	+	+	Glucose
Lactose	-	d	+	+	+	-	-	-	-	+	+	+	+	Lactose
Sucrose	+	+	+	+	+	[-]	+	-	-	+	+	+	+	Sucrose
Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	Gas
H₂S	-	-	-	-	-	-	-	-	-	-	-	-	-	H₂S
Mannose	[-]	d	+	+	+	[-]	[-]			+	+	+	+	Mannose
Mannitol				+	+	-	+			+	-	[-]	-	Mannitol
Raffinose	-	-	+	-	+	-	-			-	-	-	+	Raffinose
Trehalose				+	-	[-]	[-]			+	-	+	d	Trehalose
O/F	FA	OA	OA	FA	FA	OA	OA	OA	OA	FA	FA	FA	FA	O/F

FA facultative anaerobe

OA obligate aerobe

+ 90% or greater positive

- 90% or greater negative

d 26-75% of strains are positive

[+] 76-89% positive

[-] 76-89% negative