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**UNKNOWNNS LIST AND TEST RESULTS - FALL 2021 VERSION (DO NOT USE OLDER VERSIONS, AS CHANGES HAVE BEEN MADE)**

Species	Gram	Shape	Arrange	Gelatin	6.5% NaCl (broth)	7% NaCl (agar)	Urease	Nitrate	Catalase	PR-Glucose	PR-Lactose	PR-Sucrose	PR-Mannitol	PR-Inulin	Growth on EMB	Pigment	Oxidase	Hemolysis	Indole	Methyl Red	Vogues Proskauer	Citrate	Esculin	TSI (H <sub>2</sub> S)	Capsules	Motility	Species
<i>Citrobacter freundii</i>	G-	Bacillus	Single/Pairs	-	-	-	V	+	+	AG	AG	AG	AG	ND	ND	-	-	ND	(-)	+	-	+	ND	V	ND	+	<i>Citrobacter freundii</i>
<i>Enterobacter aerogenes</i>	G-	*Bacillus	Single	-	-	-	-	+	+	AG	AG	AG	AG	ND	ND	-	-	ND	-	-	+	+	+	-	ND	+	<i>Enterobacter aerogenes</i>
<i>Enterobacter cloacae</i>	G-	*Bacillus	Single	-	V	-	V	+	+	AG	AG	AG	AG	ND	ND	-	-	ND	-	-	V	+	V	-	ND	+	<i>Enterobacter cloacae</i>
<i>Escherichia coli</i>	G-	Bacillus	Single	-	-	-	-	+	+	AG	AG	V	AG	ND	ND	-	-	ND	+	+	-	-	V	-	ND	V	<i>Escherichia coli</i>
<i>Proteus mirabilis</i>	G-	Bacillus	Single	+	-	-	+	+	+	AG	-	-	-	ND	ND	-	-	ND	-	+	V	V	-	+	ND	+	<i>Proteus mirabilis</i>
<i>Proteus vulgaris</i>	G-	Bacillus	Single	+	-	-	+	+	+	A(G)	-	A(G)	-	ND	ND	-	-	ND	+	+	-	-	V	+	ND	+	<i>Proteus vulgaris</i>
<i>Pseudomonas aeruginosa</i>	G-	Bacillus	Single	+	-	-	+	+	+	-	-	-	-	ND	ND	+	+	ND	-	-	+	+	ND	-	ND	+	<i>Pseudomonas aeruginosa</i>
<i>Pseudomonas fluorescens</i>	G-	Bacillus	Single	-	-	-	-	-	+	-	-	-	-	ND	ND	V	+	ND	-	-	(+)	(+)	ND	-	ND	+	<i>Pseudomonas fluorescens</i>
<i>Salmonella typhimurium</i>	G-	Bacillus	Single	+	-	-	-	+	+	AG	-	-	AG	ND	ND	-	-	ND	+	+	+	+	-	+	ND	+	<i>Salmonella typhimurium</i>
<i>Shigella dysenteriae</i>	G-	Bacillus	Single	-	-	-	-	+	+	A	-	-	A	ND	ND	-	-	ND	+	+	-	-	-	-	ND	-	<i>Shigella dysenteriae</i>
<i>Shigella flexneri</i>	G-	Bacillus	Single	-	-	-	-	+	+	A(G)	-	-	A(G)	ND	ND	-	-	ND	ND	+	-	-	-	-	ND	-	<i>Shigella flexneri</i>
<i>Enterococcus faecalis</i>	G+	Coccus	Chains	V	+	-	ND	-	-	A	A	A	A	-	ND	-	-	α or -	-	ND	ND	ND	+	-	ND	-	<i>Enterococcus faecalis</i>
<i>Lactococcus lactis</i>	G+	*Coccus	Single/Pairs/Chains	-	-	-	ND	ND	-	A	A	V	V	V	+	-	-	α or -	ND	+	-	-	+	-	-	-	<i>Lactococcus lactis</i>
<i>Micrococcus luteus</i>	G+	Coccus	Clusters	+	-	-	+	-	+	-	-	-	-	ND	ND	+	+	-	-	ND	ND	-	-	-	ND	-	<i>Micrococcus luteus</i>
<i>Micrococcus varians</i>	G+	Coccus	Clusters	+	V	V	+	+	+	A	-	ND	-	ND	ND	+	-	-	ND	ND	ND	+	-	-	ND	-	<i>Micrococcus varians</i>
<i>Staphylococcus aureus</i>	G+	Coccus	Clusters	+	+	+	(+)	+	+	A	A	A	A	ND	ND	+	-	β	-	-	+	-	-	-	-	-	<i>Staphylococcus aureus</i>
<i>Staphylococcus epidermidis</i>	G+	Coccus	Clusters	+	+	+	+	(+)V	+	A	V	A	-	ND	ND	-	-	α or -	-	-	+	-	-	-	-	-	<i>Staphylococcus epidermidis</i>
<i>Streptococcus agalactiae</i>	G+	Coccus	Chains	-	-	-	-	ND	-	A	A	A	-	-	ND	-	-	β	ND	ND	+	ND	-	-	+	-	<i>Streptococcus agalactiae</i>
<i>Streptococcus bovis</i>	G+	Coccus	Chains	-	-	-	-	ND	-	A	A	A	(A)	+	-	-	-	α or -	ND	ND	V	ND	+	-	+	-	<i>Streptococcus bovis</i>
<i>Streptococcus mitis</i>	G+	Coccus	Chains	-	-	-	+	ND	-	A	A	A	-	-	ND	-	-	α	ND	ND	-	ND	-	-	+	-	<i>Streptococcus mitis</i>
<i>Streptococcus pneumoniae</i>	G+	Coccus	Chains	-	-	-	-	ND	-	A	A	A	-	+	ND	-	-	α	ND	ND	-	ND	-	-	+	-	<i>Streptococcus pneumoniae</i>
<i>Streptococcus pyogenes</i>	G+	Coccus	Chains	-	-	-	-	ND	-	A	A	A	-	-	ND	-	-	β	-	ND	-	ND	-	-	+	-	<i>Streptococcus pyogenes</i>

\* *E. cloacae*, *E. aerogenes* and *L. lactis* may instead have a COCCOBACILLUS shape

V = Variable

( ) = Variable, but more likely to be whatever is in the parentheses. For example (+) = most likely positive although can be negative.

ND = Not Determined (essentially the same as "V" variable)

**UNKNOWNNS TESTS – HELPFUL TIPS...**

This information is in addition to that found in the instructions for each test.

**Growth on EMB test: after TWO days incubation**

**Gram Stain** Note: To make a Gram-stain from a broth, it is easier to transfer cells using a sterile cotton swab rather than multiple loopfuls of broth.

**Arrangement** Note: In Gram-stains made from agar stock cultures, chains often break apart and the cells clump up and look like clusters. It is generally safer to use arrangement as a guide ONLY, and NOT to use it to eliminate any of your bacteria.

**Gelatin** Note: Use **room temperature** incubation.

**6.5% NaCl** Note: Are BROTH cultures. The presence of turbidity is a positive result. The absence of turbidity is a negative result.

**7% NaCl** Note: Are AGAR PLATES. The presence of isolated colonies (especially in sectors 2 or 3 of a streaked plate) is a positive result. The absence of isolated colonies is a negative result.

**Urease** Note: Urease broths are located in the vegetable bin in the culture media refrigerator in the front right corner of the lab room (please ask instructor before obtaining them).

**PR-Glucose** Note: PR-Carbohydrate Broths contain a single fermentable carbohydrate. PR-Glucose Broths contain Glucose.

**PR-Lactose** Note: PR-Carbohydrate Broths contain a single fermentable carbohydrate. PR-Lactose Broths contain Lactose.

**PR-Sucrose** Note: PR-Carbohydrate Broths contain a single fermentable carbohydrate. PR-Sucrose Broths contain Sucrose.

**PR-Mannitol** Note: PR-Carbohydrate Broths contain a single fermentable carbohydrate. PR-Mannitol Broths contain Mannitol.

**PR-Inulin** Note: PR-Carbohydrate Broths contain a single fermentable carbohydrate. PR-Inulin Broths contain Inulin. Inulin broths do not contain Durham tubes (that catch gas) because the organisms that are typically tested for Inulin fermentation ability do not produce gaseous fermentation by-products.

**Pigment** Note: A positive pigment result should show obvious color production. **That is, even before you knew that there was such a thing as a pigment test, you already noticed that your organism was producing a color. If you have to look to see if your organism produces a pigment, that is a negative result.** Note also that a negative pigment result is even less reliable than most negative results.

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