MICROBIOLOGY 102 – THE *ENTEROBACTERIACEAE*

A "virtual" enteric plating demonstration that can go along with discussion of MacConkey Agar is at this web address: http://www.jlindquist.com/generalmicro/dfentericplate4.html

GENERAL CHARACTERISTICS OF THE "ENTERICS"

with comparison to *Pseudomonas* and the "lactics" (Experiment 12)

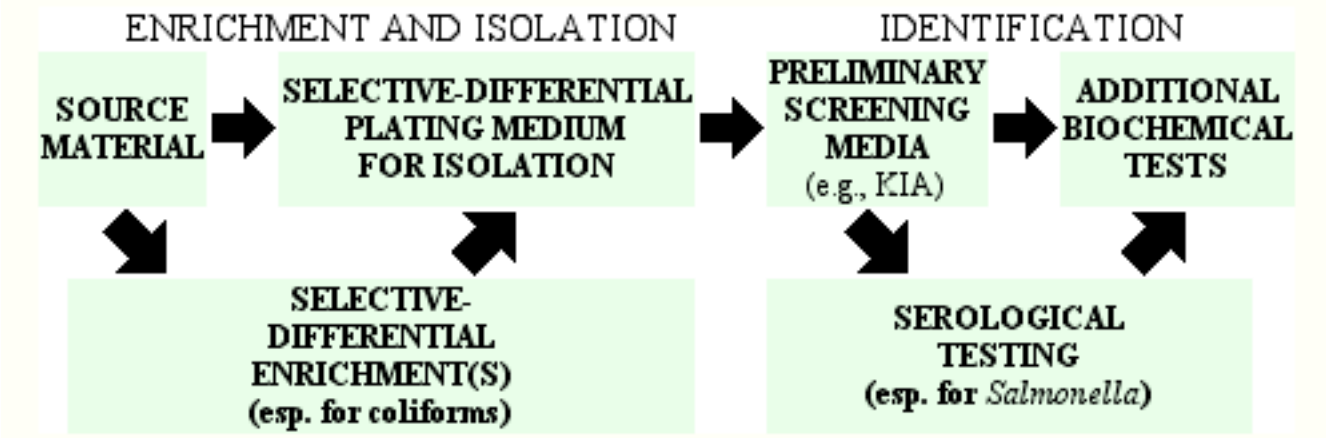
PARAMETER	**ENTERIC BACTERIA** (or "enterics") Family *Enterobacteriaceae*	***Pseudomonas*** (a strictly aerobic non-enteric which tends to be isolated on the same media)	**LACTIC ACID BACTERIA** (or "lactics")
Gram Reaction	–	–	+
Morphology*	rod (generally short)	rod	coccus or rod
Carbo-Metabolic Type	chemoheterotroph	chemoheterotroph	chemoheterotroph
Catabolism**	aerobic respiration; anaerobic respiration (with nitrate); fermentation	aerobic respiration; often anaerobic resp. (with nitrate)	fermentation
Oxygen Relationship***	facultative anaerobe	strict aerobe	aerotolerant anaerobe
Catalase Reaction	+	+	–
Glucose Fermentation	+	–	+
Fermentation Types	mixed acid & butanediol	none	homofermentative & heterofermentative
Oxidase Reaction	–	+	–
Motility	usually +	+	–
Need for Growth Factors	none or minimal	none or minimal	generally extensive
Primary Consideration for Isolation	selection against Gram+ bacteria	selection against Gram+ bacteria	azide tolerance with **aerobic** incubation

* The enterics generally shorten during extended growth of the culture such that the rod-shaped cells assume an oval shape. In the lactic acid bacteria, the cocci sometimes elongate and appear oval-shaped!

** Recall the **five general types of catabolism:** Aerobic respiration, anaerobic respiration, fermentation, anoxygenic phototrophy and oxygenic phototrophy.

*** Recall how "**oxygen relationship**" relates to **glucose fermentation** and the **catalase reaction**.

GENERALIZED ENTERIC ISOLATION PROCEDURE



REVIEW OF FEATURES WHICH MAY BE NOTED FOR MANY DIFFERENTIAL MEDIA

AEROBIC OR ANAEROBIC	SUBSTRATE	MICROBIAL ACTIVITY	REACTION	SOME EXAMPLES
AEROBIC	various **amino acids** in peptones, etc.	**deamination***	**alkaline**	MacConkey Agar, O/F Medium, Fermentation Broth, KIA, MIO
ANAEROBIC	specific **sugar** in **small** amount	**fermentation**	**acid**	KIA, Lysine Broth and MIO (glucose for all 3)
	specific **sugar** in **large** amount**	**fermentation**	**ACID**	MacConkey Agar, O/F Medium, Fermentation Broth, KIA
	specific **amino acid** in **large** amount	**decarboxylation**	**ALKALINE**	MIO (ornithine), Lysine Broth
	thiosulfate	**reduction** with **H_2S formation**	**black color** (with Fe)	KIA, Microbiology 102's special Modified MacConkey Agar

* All enterics (like most common chemoheterotrophs) will deaminate amino acids in peptones, yeast and beef extract, and similar materials.

** A relatively large amount of glucose along with a much smaller than usual amount of peptone accounts for the ability to detect the **small amount of acid associated with respiration of the sugar** (glucose) in Glucose O/F Medium which can be seen for certain gram-negative, strictly aerobic organisms like *Pseudomonas*.

RELEVANT THOUGHT QUESTION NO. 1

You wish to exploit certain properties of the difficult-to-isolate bacterium ***Excalibacterium*** (an enteric) so that you can efficiently detect and isolate it from samples which are highly-contaminated with other enterics. You decide to start with **MacConkey Agar** which you know contains **lactose** as the only fermentable sugar. **Peptone** is another medium ingredient which you recall; it contains a mixture of various amino acids – none in any especially high amount. Following is a table showing important organisms to consider in this situation:

genus	fermentation of					decarboxylation of	
	glucose	maltose	lactose	sucrose	mannitol	lysine	arginine
Edwardsiella	+	+	–	–	–	+	–
Aquamonas	+	+	–	–	–	+	+
Excalibacterium	+	–	–	+	–	+	–
other enterics	+	+	+ or –	+ or –	+ or –	+ or –	+ or –

a. On MacConkey Agar, what would you expect the net pH reaction would be for any of the three genera specifically listed on the table above? (Circle one) **ACID ALKALINE**

b. As these three genera do not ferment or respire lactose, how can they grow on MacConkey Agar? (Consider a likely source of energy and how they might utilize it.)

c. What would be the best choice for a sugar to add to MacConkey Agar which will assist greatly in the differentiation of *Excalibacterium* colonies from the others on the table? (Circle one) **GLUCOSE MALTOSE SUCROSE MANNITOL**

d. If lysine were to be included in the medium in a relatively large amount, what effect would this have on the pH reaction associated with *Excalibacterium* colonies? (Circle one)

MORE ACIDIC MORE ALKALINE

RELEVANT THOUGHT QUESTION NO. 2

You are in an enteric lab out in the real world, and you are picking colonies from plates of selective-differential isolation media for further testing. These plates had been inoculated with environmental samples, and we expect a variety of enterics to be present. We also expect some colonies of that pesky *Pseudomonas* to be present also.

Now, the organism you are specifically after is ***Sorgobacter***, an enteric with one or more characteristics that allow it to be differentiated from all other enterics – as seen by the reactions in the following table:

organism	fermentation of sugars:					decarboxylation of amino acids:		H_2S production
	glucose	fructose	galactose	lactose	mannitol	arginine	lysine	
Sorgobacter	+	–	+	–	–	–	+	–
other enterics	+	+	+	+or–	+or–	+or–	+or–	+or–
Pseudomonas	–	–	–	–	–	+or–	?	–

As part of the usual enteric isolation routine, you plan on picking colonies into a screening medium, such as Kligler Iron Agar (KIA) before doing a lot of specific tests. But before you do that, you decide you have time to make a modification of KIA that will allow you to decide whether you have *Sorgobacter* – just from the appearance of the modified KIA after incubation.

a. You know that KIA already has two sugars in it – lactose and glucose – and the glucose is present in a relatively small amount. Which one of the following sugars would you add to the ingredients in KIA (in a relatively large amount) such that *Sorgobacter* will look different from all of the other enterics and also *Pseudomonas*? Fructose, galactose or mannitol?

b. Briefly describe the appearance of *Sorgobacter* growing in this modified KIA. That is, would you expect an acid or alkaline reaction in the aerobic (slant) region? And what about the anaerobic region (i.e., the "butt")?

c. What would be the pH reaction in the aerobic (slant) region for all the other enterics?

d. If (for whatever reason) you were to add one of the two amino acids to the medium, which one could allow *Sorgobacter* to show an alkaline reaction in the anaerobic (butt) region?

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