THE USE OF CLARK AND LUBS INDICATORS FOR THE DETECTION OF ACID PRODUCTION BY THE COLON-TYPHOID GROUP.

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The incorporation, into a medium, of heat resisting indicators is one of the common methods practiced for detecting and determining acid production by bacteria.[1] Clark and Lubs indicators lend themselves especially well to this kind of work because they are heat resisting; they do not inhibit bacterial growth; they resist the action of bacteria; and with a number of them, the virage point is at or near the acid concentration of ordinary stock media. The purpose of the following experiments was to differentiate between the various members of the colon-typhoid group by means of their action on various sugars. No attempt was made to measure the amount of acid produced in each case.

The medium used was a 3 per cent nutrient agar made with .3 per cent Liebig's beef extract, 2 per cent Difco peptone and .5 per cent sodium chloride. The ingredients were mixed and autoclaved at ten pounds for one hour, filtered and the reaction adjusted to slight pink to phenol red (Ph=6.8—7.5). Then the bulk was divided into workable portions and .1 per cent of the desired sugar added to each. This was further divided into three portions and .002 parts of indicator added to each portion. The prepared media was then tubed, sterilized in the autoclave at ten pounds for ten minutes and slanted. Care was taken in slanting to allow for a butt in each tube of at least one inch below the bottom of the slant.[2] After solidification the tubes were ready for inoculation.

The indicators used were brom cresol purple, brom thymol blue, and phenol red. The dyes were all made up in 1 per cent alcoholic solutions which were used for stock solutions. In making dilutions .1 cc. of this 1 per cent stock solution was added to a graduate and media poured into it up to the 50 cc. mark. This gave a dilution of 1 part of dye to 2,000 parts of media. By this method the dye readily mixed with the media and did not require further mixing.

The sugars included dextrose, lactose, sucrose, maltose, raffinose, mannose, dulcitol, xylose, rhamnose, arabinose, levulose, and galactose. All these sugars went into solution very readily if added directly to the hot medium. Some of these are classified as rare sugars and would not be practicable for class room work if used in 1 per cent quantities.

The cultures used were the stock cultures used for laboratory work including *B. coli*, *B. typhosum*, *B. dysenteriae*, *B. paratyphosum* A,

[1] H. R. Baker. Substitution of B.T.B. for Litmus in Routine Laboratory Work. Jour. Bact. Vol. VII. 2.

J. Bronfenbrenner, M. J. Schlesinger and D. Soletsky. On Methods of Isolation and Identification of the Members of the Colon-Typhoid Group of Bacteria. The Study of Bactericidal action of C. R. Indicator. Jour. Bact. Vol. V. 1.

[2] H. J. Conn and G. J. Hucker. The Use of Agar Slants in detecting Fermentation. Jour. Bact. Vol. V. 4.

"Proc. 38th Meeting, 1922 (1923)."

B. paratyphosum B, and *B. enteritidus.* These cultures were carried on stock agar and transferred every twenty-four hours till ready for use.

Inoculations were made in duplicate tubes with a straight platinum wire, inoculating the surface of the slant first and then making a stab in the butt from the bottom of the slant to the bottom of the tube. In reading the tubes the slant and stab were read separately. The stab gave the acid production under anaerobic conditions while the slant gave the acid change under aerobic conditions.

All tubes were incubated at 37° C. for 24 hours.

The following tables give the results obtained.

TABLE 1. 24 Hour Growth. .1 Per cent Arabinose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	yellow	yellow	yellow
	Butt	Color	yellow	green	yellow	yellow	yellow	yellow
		Gas	+	—	—	+ +	+	+ +
B. C. P.	Slant	Color	purple	purple	purple	yellow	yellow	yellow
	Butt	Color	yellow	purple	purple	yellow	yellow	yellow
		Gas	+ + +	—	—	+ + +	+ +	+ +
P. R.	Slant	Color	pink	pink	pink	yellow	yellow	yellow
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+ + +	—	—	+ + +	+ + +	+ + +

TABLE 2. 24 Hour Growth. .1 Per cent Dextrose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	yellow	yellow	yellow	yellow	yellow	yellow
	Butt	Color	yellow	green	green	yellow	yellow	green
		Gas	+	—	—	+ +	+ +	+
B. C. P.	Slant	Color	yellow	yellow	purple	yellow	yellow	yellow
	Butt	Color	red	purple	purple	red	yellow	purple
		Gas	+	—	—	+ +	+ +	+
P. R.	Slant	Color	yellow	yellow	yellow	yellow	yellow	yellow
	Butt	Color	yellow	pink	pink	yellow	pink	pink
		Gas	+	—	—	+ +	+ +	+

TABLE 3. 24 Hour Growth. .1 Per cent Duleitol.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	green	green	green
	Butt	Color	green	green	green	green	green	green
		Gas	—	—	—	—	+	+
B. C. P.	Slant	Color	purple	purple	purple	purple	purple	purple
	Butt	Color	purple	purple	purple	purple	purple	purple
		Gas	—	—	—	—	+	+
P. R.	Slant	Color	pink	pink	pink	pink	pink	pink
	Butt	Color	yellow	yellow	yellow	pink	yellow	yellow
		Gas	—	—	—	—	+	+

TABLE 4. 24 Hour Growth. .1 Per cent Galactose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	yellow	yellow	green	yellow	yellow
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+ + +	—	—	+	+ + +	+ +
B. C. P.	Slant	Color	purple	yellow	green	purple	yellow	yellow
	Butt	Color	yellow	yellow	purple	red	yellow	yellow
		Gas	+ + +	—	—	+	+ + +	+ +
P. R.	Slant	Color	pink	yellow	yellow	yellow	yellow	yellow
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+ + +	—	—	+	+ + +	+ + +

TABLE 5. 24 Hour Growth. .1 Per cent Lactose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	yellow	green	green	green	green	green
	Butt	Color	yellow	green	green	green	green	green
		Gas	+ +	—	—	—	—	—
B. C. P.	Slant	Color	yellow	purple	purple	purple	purple	purple
	Butt	Color	yellow	purple	purple	purple	purple	purple
		Gas	+ +	—	—	—	—	—
P. R.	Slant	Color	yellow	pink	pink	pink	pink	pink
	Butt	Color	yellow	pink	pink	pink	pink	pink
		Gas	+ +	—	—	—	—	—

TABLE 6. 24 Hour Growth. .1 Per cent Levulose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	yellow	green	yellow	green	green
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+ + +	—	—	+ + +	+ + +	+ + +
B. C. P.	Slant	Color	purple	yellow	purple	yellow	purple	purple
	Butt	Color	yellow	yellow	purple	yellow	yellow	yellow
		Gas	+ + +	—	—	+	+ + +	+ + +
P. R.	Slant	Color	pink	yellow	pink	yellow	pink	pink
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+ + +	—	—	+ + +	+ + +	+ + +

TABLE 7. 24 Hour Growth. .1 Per cent Maltose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	green	green	green
	Butt	Color	yellow	yellow	green	yellow	yellow	yellow
		Gas	+ + +	—	—	+ + +	+ + +	+ + +
B. C. P.	Slant	Color	purple	purple	purple	purple	purple	purple
	Butt	Color	yellow	yellow	purple	yellow	yellow	yellow
		Gas	+ + +	—	—	+ + +	+ + +	+ + +
P. R.	Slant	Color	pink	pink	pink	pink	pink	pink
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+ + +	—	—	+ + +	+ + +	+ + +

TABLE 8. 24 Hour Growth. .1 Per cent Mannose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	green	green	green
	Butt	Color	yellow	yellow	yellow	green	green	green
		Gas	+	—	—	+	+	+
B. C. P.	Slant	Color	purple	purple	purple	purple	purple	purple
	Butt	Color	purple	yellow	purple	purple	purple	green
		Gas	+	—	—	+	+	+
P. R.	Slant	Color	pink	pink	pink	pink	pink	pink
	Butt	Color	yellow	yellow	yellow	yellow	grey	grey
		Gas	+	—	—	+	+	+

TABLE 9. 24 Hour Growth. .1 Per cent Raffinose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	green	green	green
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	—	—	—	—	—	—
B. C. P.	Slant	Color	purple	purple	purple	purple	purple	purple
	Butt	Color	purple	purple	purple	purple	purple	purple
		Gas	—	—	—	—	—	—
P. R.	Slant	Color	pink	pink	pink	pink	pink	pink
	Butt	Color	yellow	yellow	yellow	yellow	grey	grey
		Gas	—	—	—	—	—	—

TABLE 10 24 Hour Growth. .1 Per cent Rhamnose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	green	green	green
	Butt	Color	green	green	green	yellow	yellow	yellow
		Gas	+	—	—	+	—	+
B. C. P.	Slant	Color	purple	purple	purple	purple	purple	purple
	Butt	Color	green	purple	purple	green	purple	yellow
		Gas	+ +	—	—	+ +	—	+ +
P. R.	Slant	Color	pink	pink	pink	pink	pink	pink
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+ +	—	—	+	—	+ +

TABLE 11. 24 Hour Growth. .1 Per cent Sucrose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	green	green	green
	Butt	Color	green	yellow	lt. green	green	green	green
		Gas	—	—	—	—	—	—
B. C. P.	Slant	Color	purple	purple	purple	purple	purple	purple
	Butt	Color	purple	purple	purple	purple	purple	purple
		Gas	—	—	—	—	—	—
P. R.	Slant	Color	pink	pink	pink	pink	pink	pink
	Butt	Color	yellow	yellow	yellow	yellow	grey	grey
		Gas	—	—	—	—	—	—

TABLE 12. 24 Hour Growth. .1 Per cent Xylose.

			Colon	Typhoid	Dysentery	Para A	Para B	Enteritidus
B. T. B.	Slant	Color	green	green	green	green	green	green
	Butt	Color	yellow	green	green	green	yellow	yellow
		Gas	+	—	—	—	+	+
B. C. P.	Slant	Color	purple	purple	purple	purple	purple	purple
	Butt	Color	purple	purple	purple	purple	purple	purple
		Gas	+	—	—	—	+	+
P. R.	Slant	Color	pink	pink	pink	pink	pink	pink
	Butt	Color	yellow	yellow	yellow	yellow	yellow	yellow
		Gas	+	—	—	—	+	+

Summarizing these tables it will be found that all three indicators may be used in media for the detection of acid. Phenol red is the most sensitive, as might be expected, then brom thymol blue and finally brom cresol purple. The last two proved very satisfactory and could almost be used interchangeably. The amount of acid produced is the determining factor in each case so that we might expect different results with different indicators. For example, typhoid and dysentery may be differentiated by their action on arabinose with brom thymol blue but not with brom cresol purple. Not enough acid has been produced to bring about a change with brom cresol purple. On the other hand, these same organisms may be differentiated by their action on mannose and galactose with brom cresol purple but not with brom thymol blue. In this case the action has gone too far to be detected with brom thymol blue.

All the sugars used were fermented by one or more organisms. Three of these, dulcitol, raffinose and sucrose were fermented with very little or no gas so that for this purpose they are of little or no use.

Arabinose will divide the group into four parts. Colon, typhoid and dysentery stand out by themselves while para A., para B., and enteritidus remain undifferentiated.

Dextrose is the most easily fermented of all the sugars used. Typhoid, dysentery and enteritidus stand out as distinct individuals while the other three show no difference in their reactions.

Galactose breaks up the group into five parts, para B. and enteritidus in one group and the other four as separate individuals.

Lactose is fermented only by colon.

Levulose gives different reactions for typhoid, dysentery, and para A. while the same reaction is given for colon, para B. and enteritidus.

Maltose is fermented by typhoid but not by dysentery, thereby distinguishing between these two. The others all give like reactions.

Like maltose, mannose will separate typhoid and dysentery but will further divide the remaining four into three groups, colon and enteritidus as separate individuals and the other two undifferentiated.

Rhamnose will distinguish between colon, para A., para B., and enteritidus. Typhoid and dysentery are undifferentiated.

Xylose gives one reaction for colon, para B. and enteritidus and another for typhoid, dysentery and para A.