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UTILIZATION OF CHINIC ACID IN THE DIFFERENTIATION OF THE COLON-AEROGENES GROUPS

BUFORD H. BUTCHER

Beijerinck in 1911 called attention to the fact that some strains of *Bact. aerogenes* produce from chinic acid and a ferric salt, a red to black coloration under aerobic conditions. Strains of *Bact. coli*, he stated, do not color the medium under similar conditions. Under anaerobic conditions in solutions of chinate salt, a fermentation may occur with some members of the aerogenes group, whereby carbonic, acetic and propionic acids are formed. At the Iowa State College laboratory an attempt was made to duplicate this anaerobic type of fermentation by organisms of the coli-aerogenes group but without success. Both Smith and Durham tubes were used and no gas was detected. However, the coloration produced by *Bact. aerogenes* when grown on chinate medium was found to be pronounced. This color is soluble and diffuses through the medium, either liquid or solid. The suggestion from this article of Beijerinck's presented an opportunity for the development of a possible differential test for coli and aerogenes strains.

The laboratory methods usually employed for the differentiation of the coli and aerogenes group are the Voges Proskauer and the methyl red reactions. Both of these are classified as color reactions and they require a medium consisting of dextrose, peptone, and phosphate. If *Bact. aerogenes* is grown on this medium and then a strong alkali added, a pink color slowly develops due to the presence of acetyl methyl corbinol produced by the organism. This is the Voges Proskauer reaction. When *Bact. coli* is grown on the above medium it becomes acid to methyl red which is used as an indicator. These two tests should check against each other without any discrepancy, that is, when Voges Proskauer is positive in case of the aerogenes group, the same group when tested with methyl red should be distinctly alkaline, but when a member of the colon group is being tested the Voges Proskauer is negative and the methyl red reaction is acid.

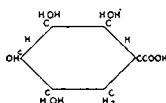
There are other tests which may be considered supplementary to the Voges Proskauer and methyl red reactions. Thus in con-

sidering the coli group the gas ratio CO₂-H₂ is nearly 1:1 and indol usually positive. In dealing with aerogenes group, the gas ratio CO₂-H₂ is 2:1 and indol usually negative. Another interesting differential test might be mentioned here. S. A. Koser (1922) states that *Bact. aerogenes* will produce abundant growth in a medium in which a sodium, potassium, or an ammonium salt of citric acid is the sole source of carbon. This citric acid medium will not support the growth of *Bact. coli*.

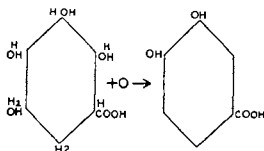
This work on the chinic acid medium and its coloration was undertaken with the belief that it might prove to be a useful test which could be used in connection with the differential methods just mentioned. The experimental work necessary for carrying out this test is simple, direct, and convenient. The medium used is as follows:

H ₂ O.....	100.0	cc
K ₂ HPO ₄	0.05	g
NH ₄ Cl or peptone.....	0.05	g
Calcium chinate.....	1.00	g
Ferric chloride.....	0.01	g

Chinic acid is in chemical terms, hexa-hydro-tetra-oxy benzoic acid having the probable formula:



The calcium salt is made by the simple process of neutralization. The minimum quantity of the calcium salt to be used for satisfactory results is 0.5% to 1.0%, although a rather distinct coloration is obtained by using as low as 0.2%. It has been found much preferable to use agar agar slants or plates rather than the liquid medium. This is quite reasonable since the coloration, in case of *Bact. aerogenes*, is due to an oxidizing action which may be indicated by the equation:



Thus protocatechoic acid is an oxidation product of chinic acid and it is this di-hydroxy benzoic acid which is responsible for the coloration with an iron salt. This in principle is nothing but the ordinary phenol test. Obviously more favorable aerobic con-

ditions are obtained on the surface of solid medium than in liquid cultures. After inoculation the chinate medium is incubated, preferably at 30° and within 24 hours some strains of *Bact. aerogenes* produce a distinctly dark color on the surface of slants or plates or throughout a liquid culture. Other strains which do not give a distinct Voges Proskauer reaction require three or four days of incubation to develop a pronounced coloration.

A total of forty-three known organisms were tested on the chinate medium, eighteen of them being members of the aerogenes group, the other twenty-five belonging to the species *Bact. aerogenes*, five belonging to the species *Bact. oxytocom*, five were *Bact. cloacae*, and two were *Bact. levans*. The *Bact. aerogenes* and *Bact. oxytocom* organisms checked well with the Voges Proskauer reaction. The *Bact. cloacae* and *Bact. levans* tried produced no coloration. This experimental evidence indicates the possibility of differentiating between organisms even within the aerogenes group as well as between the coli and aerogenes groups. Of the twenty-five strains of coli tested none gave any coloration to the chinate medium.

It was thought that since the coloration in the medium is a result of an oxidizing ferment or enzyme, the addition of a source of oxygen to the medium in the form of nitrate might hasten the appearance of the dark color. The presence of the nitrate produced no appreciable affect.

This differential test for the coli group and aerogenes group is suitable for routine work or even work of a more exacting nature. After inoculation and placing in the incubator the cultures require no further attention until one examines them for results.

A medium containing as much as 1.0% of calcium chinate may be preserved for many weeks without any decomposition or discoloration, but in higher concentrations of 10.0% or more the medium will darken after three or four weeks especially if kept at 37°.

In summation, the calcium chinate test has these points to recommend it: reliability, simplicity, convenience, and a saving of time when compared with the standard Voges Proskauer method, moreover the cost of the chinate is not prohibitive even for routine work.

REFERENCES

Beijerinck. Pigments as products of oxidation by bacterial ac-

tion. Proc. Sect. Sci. Kon. Akademy von Wetenschappen, Amsterdam, vol. 13, p. 1066-1077. 1911.

Reprint. Beijeinck. Verzamelde Geschriften, vol. 5, p. 1-10. 1911.

Koser. Utilization of the salts of organic acids by the colon-aerogenes group. Jour. Bact., vol. 8, p. 493-520. 1923.

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