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The utilization of simple sugars by Alcaligines fecalis

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THE UTILIZATION OF SIMPLE SUGARS BY ALCALIGINES FECALIS

by

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Introduction

Alcaligines fecalis has been and still is considered carbohydrate inert. That is, during growth in sugar broths it produces no perceptible acid or gas reaction. According to the classification in Bergey's Manual this organism is .5 by 1-2 microns in size, occurring either singly or in pairs, occasionally in long chains. It is motile by peritrichous flagella, and stains gram negatively. On gelatin plates it produces circular, grayish, translucent colonies. In gelatin stabs there is growth along the line of inoculation as well as a gray surface growth, but there is no liquefaction. On agar plates the colonies are translucent with opaque centers and undulate margins. On plain agar slants the streak is whitish-gray, glistening, and opalescent, and also has an undulate margin. Growth in broth produces turbidity throughout with a thin pellicle on the surface and a viscid sediment. It is alkaline in litmus milk and gives off ammonia. On potato there is from scanty to abundant growth, yellowish to brownish in color. It does not form indol nor reduce nitrates, nor does it produce acid or gas in the carbohydrates. It is an odorless acrobe, facultative anaerobe, growing at an optimum temperature of 37° C. Its normal habitat is in the intestinal tract of man.

The aim of the problem was to attempt to demonstrate conclusively the ability of Alcaligines fecalis to metabolize

some simple sugars, and, if the organisms did show a tendency toward this utilization, the problem would then be to test for possible end products.

Historical Review

Literature pertinent to this problem is extremely scarce. After an extensive search the author found references which both support and deny the probability of carbohydrate metabolism by Alcaligines fecalis. To quote B. L. Monias:[7]

> "Among the cultures isolated by different authors under the name of Bacterium fecale alcaligines (1-15) two morphologically as well as biologically different groups could be distinguished. The organisms of the first group which include Petruschy's original culture isolated from decomposed beer, are motile with peritrichous flagella. Cultures of this group ferment all monosaccharides and especially the hexoses. Some of the strains form acid only, others develop gas from carbohydrates. Some of these cultures ferment monoses only, others act on the bioses (disaccharides). The organisms of the second group are also motile, have polar flagella, and do not ferment any carbohydrates.
>
> "The first of these two groups is related to the group of Bacterium coli. Their fermentation power is much less than that of the coli group and to a great extent no acid can be produced from lactose. Alcaligines fecalis, Petruschy named according to the nomenclature of the Society of American Bacteriologists, typifies this species, and is described by Bergey's as being 'motile by means of peritrichous flagella'. The further remark that it does not form acid or gas in carbohydrate mediums does not agree with my findings on cultures examined. It might be that if solid mediums under purely aerobic conditions were used, different results would be obtained.
>
> "The method used in this examination was that of anaerobic culture in liquid medium (Congo red broth)."

Since those organisms of the first group are not now

considered as Alcaligines fecalis they must obviously be excluded from present consideration. The results of Monias imply the utilization of carbohydrates by Alcaligines fecalis and are in agreement with this experimental work.

According to M. H. Soule[11] in his work with the respiration of the Abortus-Melitensis group, the

> "addition of 2% glucose to the medium favored the growth of all cultures and exerted a sparing action on the decomposition of the proteins and implying a utilization of glucose."

On the other hand, Kendall, Friedman, and Ishikawa[4] state in their work with "resting" Alcaligines fecalis, there is a utilization of lactic acid and alanine, but that there is no action on dextrose, and but a slight action on pyruvic acid. This statement is in direct opposition to the conclusion of Soule.

In work by Kendall, Day, and Walker[3] it is proposed that Alcaligines fecalis does not produce acid in glucose and that there is no sparing action of this sugar for protein. This result, they contend, is manifested by the formation of as much ammonia in the sugar broth as in the sugar-free broth, when both were kept under the same conditions. Their results are in further opposition to Soule.

In these latter references it must be considered that conclusions were drawn on the basis of protein metabolism and not on the basis of actual sugar metabolism. No mention was made of a quantitative sugar analysis. Such results are

not too far reaching since these organisms do not necessarily have to belong in the category of "protein sparing" types.

Experimental

H. D. Dakin[2] asserts that the principal path of glucose metabolism in the animal body is not always from glucose through glucuronic acid, d-saccharic acid, oxalic acid, and finally carbon dioxide and water. This conclusion, according to Dakin, is because it has long been recognized that hexoses could by a variety of processes be converted into substances containing three carbon atoms. He mentions that Embden has shown lactic acid may give rise to glycogen in the normal animal, and when given to a diabetic animal it is largely converted into glucose. Dakin's conclusion is,

> "Lactic acid must therefore be regarded as one of the most important substances concerned with the intermediate metabolism of the carbohydrates."

Experiments were then begun on the presumption that if Alcaligines fecalis did use sugars, lactic acid may be produced in detectable amounts. Growth tests were run by using the four common sugars given below. These were made up to a .5% concentration in Munter's solution.[5] This solution contains,

$MgSO_4$	0.5 gm.
NaCl	0.5 gm.

$CaCl_2$ . . . . . 0.2 gm.

K_2HPO_4. . . . . 1.0 gm.

H_2O (distilled) . . . .1000 c.c.

For each 100 c.c. of this solution .025 gm. of NH_4NO_3 were added as a nitrogen source. Two hundred c.c. of the resulting solution were poured into each of four 250 c.c. Erlenmeyer flasks. Then 1 gm. of sucrose was added to the first flask, 1 gm. of galactose to the second, 1 gm. of lactose to the third, and 1 gm. of arabinose was added to the last. All these weighings were approximate, since the sugar determinations in later work were to be determined by titration. The resulting solutions were then divided into 100 c.c. portions. Each portion was then poured into a 250 c.c. Erlenmeyer flask and sterilized at ten pounds pressure for twenty minutes. One flask was inoculated with the organism, the other served as a sterile control. Three 100 c.c. portions of sugar-free Munter's solution were inoculated and incubated. All were incubated for three weeks at 37° C. At the end of this time turbidities appeared, slightly in arabinose and more pronounced in galactose. In sucrose the turbidity was hardly discernible. Lactose remained as clear as the controls which had no trace of turbidity. Agar slants were made from the inoculated plain Munter's solution. Good growth appeared after two days. There was no turbidity in these mixtures. This shows that the organisms remain

viable in the plain solution. The inoculated solutions were filtered through a Berkefeld filter. This method was later discarded, because rinsing for residual sugar proved too difficult and would induce a higher percentage error for quantitative work. Filtrations were finally made through No. 50 Whatman filter papers into a 100 c.c. graduate. The purpose of filtration was to remove the precipitate that settled on sterilization. The filtrate was diluted up to 100 c.c. to compensate for loss during evaporation, so as to have the same sugar concentration for initial quantitative work. In order to accomplish this dilution, 10 c.c. of distilled water were added to the original flask and flushed around in it. The water was then poured through the filter paper. This procedure was repeated until the desired 100 c.c. were obtained. The residual sugar was removed from the original flask and filter paper very efficiently by this method.

The method of Stiles, Peterson and Fred[10] was used for quantitative sugar analysis. For this work galactose and arabinose were chosen, because they are single sugars. This choice eliminated the necessity of hydrolysis with HCl before titration, a procedure that would introduce further experimental error. In each case before tests were run, either for end products or for quantitative determination of sugar, methylene blue morphology slides were made from the inoculated

media. Inoculations were also made into litmus broths of sucrose, dextrose, and lactose. These tubes were then incubated for 48 hours at 37° C. In all cases these two checks gave characteristics which were typical for Alcaligines fecalis.

To carry out quantitative sugar analyses the following solutions were used:

(a) Micro Reagent

$CuSO_4 5H_2O$	5.0 gm.
Tartaric Acid	7.5 gm.
Na_2CO_3 (anhyd.)	40.0 gm.
KI.	10.0 gm.
KIO_3	0.7 gm.
Pot. Axalate	18.4 gm.
H_2O (distilled)	1000 c.c.

(b) 1 N. H_2SO_4

(c).1 N. Na_2SO_3

(d) 33% solution of lead acetate

(e) 10% solution of NaH_2PO_4

After filtration of the media 10 c.c. were taken and diluted to 100 c.c., then 5 c.c. of the lead acetate were added to remove the chlorides, and 15 c.c. of the phosphate were added to remove the calcium. This gave a dilution of the original solution of one part in six. The whole was allowed to stand until the precipitate settled. After settling, a

5 c.c. aliquct was withdrawn, placed into a 50 c.c. Erlenmeyer flask, and diluted to 10 c.c., then 5 c.c. of the micro-reagent were added, the flask corked to prevent oxidation from the air, and placed in a boiling water bath for fifteen minutes. At the end of the boiling, the flask was cooled under running tap water, acidified with 5 c.c. of 1 N.N_2SO_4, allowed to stand one minute, and then titrated against .005 N.$Na_2S_2O_3$ until a light straw color appeared. At this point one drop of a 1% starch solution was added as an indicator, and the titration continued to the disappearance of the blue starch-iodide color.

Before running any quantitative sugar analysis, the micro-reagent must be titrated against .005 N.$Na_2S_2O_3$ so as to determine the exact amount of this reagent required by the micro-reagent. This normality is obtained by diluting the .1 N $Na_2S_2O_3$ to twenty times its original volume.

The titration may be illustrated by the following example:

Titration of blank(5 c.c.) . . . 16.65 c.c. .005 N.$Na_2S_2O_3$
" " sample(5 c.c.). . . 14.15 " " " "
Difference in c.c.of $Na_2S_2O_3$ . . 2.50
Glucose represented. . . . . .408 mg.

The difference between the c.c. of thiosulfate required for the blank and the c.c. required for the sugar solution is compared to the table of calculated results given in the

original article of Stiles, Peterson, and Fred. To get correct values for arabinose and galactose the glucose value must be multiplied by 1.06 and 1.14 respectively because the glucose value is 1.

In the chemical reaction preceding the titration we have the formation of cupric ions and iodide ions according to the following reaction,

$$2\ Cu^{+} + I_2 \rightleftharpoons 2\ Cu^{++} - 2I^{-}$$

Upon acidification the excess iodine is determined by titration with .005 N. $Na_2S_2O_3$. For complete oxidation of cuprous salts by iodine, means must be found for maintaining the concentration of cupric and iodide ions at very low values as Elbs[9] pointed out. He says that the presence of alkali oxalates more or less completely inhibit the reaction of cupric salts with soluble iodides as well as other reactions of cupric ions. Elbs interpreted this behavior by the assumption of the formation of anions containing oxalate and copper, the cations being those of the alkali.

In tables 1, 2, and 3 the exact chemical results of titrations for the three series of sugars are given. To arrive at figures in the fourth column of these tables the following formula was used:

$$\frac{\text{Sugar Represented by difference} \times 24 \times 10 \times \text{Factor}}{100}$$

In this formula, "sugar represented by difference", is similar to the example on page 8. Since 5 c.c. of the clarified solu-

tion was titrated, this represented only one-twenty-fourth of the sugar in this portion. This in turn represented only one-tenth of the sugar in the original inoculated media. Lastly, of course, the numerator must be multiplied by the sugar factor needed. The whole is divided by 100 to get mg. of sugar per c.c.

In the fifth column of each table the figure is obtained by subtracting the figure representing mg. of sugar per c.c. in the inoculated media from the figure representing mg. of sugar per c.c. in its respective control.

In the sixth column the percentage is obtained by dividing the figures of the fifth column by the mg. of sugar per c.c. of the control for the respective sugar.

Table four is a summarizing table. It will be noted in this table that the mg. of arabinose used varies between 0.424 and 0.010. Dextrose variation is between 1.114 and 0.213 mg. per c.c. while for galactose it is 2.444 and 0.602. The average for these sugars is .217, .663 and 1.523 respectively. Observation will show upon averaging, that the organisms seem to use galactose to a larger extent than they use dextrose. The second and third columns need no explanation as they are mere repetition. Figures in the fourth column are obtained by adding those of the second and third columns and dividing by two. The average percentage is obtained by taking percentages corresponding to figures in

Table 1 - Results of Titration for First Series of Sugars

Material Titrated	C. C. Reagent	Mg.Sugar per c.c. before Inoc.	Mg.Sugar per c.c. after Inoc.	Mg.Sugar Used per c.c.	Per Cent Sugar Used
Blank	16.65				
Arabinose Control	3.40	4.512	4.512		
Inoc.Arabinose No.I	4.10	4.512	4.399	0.113	2.50
" " No.II	3.60	4.512	4.502	0.010	0.22
" " No.III	3.75	4.512	4.467	0.045	0.99
Galactose Control	3.95	4.727	4.727		
Inoc.Galactose No.I	7.56	4.727	3.523	1.204	25.47
" " No.II	6.70	4.727	3.860	0.867	18.38
" " No.III	11.63	4.727	2.038	1.689	35.73

Table 2 - Results of Titration for Second Series of Sugars

Material Titrated	C. C. Reagent	Mg.Sugar per c.c. before Inoc.	Mg.Sugar per c.c. after Inoc.	Mg.Sugar per c.c. Used	Per Cent Sugar Used
Arabinose Control No. I	5.94	3.792	3.792		
Inoc.Arabinose No.I	6.1	3.792	3.765	0.027	0.71
Arab. Control No.II	5.91	3.792	3.792		
Inoc.Arabinose No.II	6.25	3.792	3.710	0.082	2.18
Arab. Control No.III	5.91	3.792	3.792		
Inoc.Arabinose No.III	6.15	3.792	3.737	0.055	1.47
Dextrose Control No.I	5.70	3.657	3.657		
Inoc.Dextrose No.I	9.30	3.657	2.546	1.111	30.38
Dextrose Control No.II	5.70	3.657	3.657		
Inoc.Dextrose No.II	6.45	3.657	3.444	0.213	5.82
Dextrose Control No.III	5.71	3.657	3.657		
Inoc.Dextrose No.III	8.71	3.657	2.712	0.945	25.84
Galactose Control No.I	6.23	4.019	4.019		
Inoc.Galactose No.I	7.90	4.019	3.417	0.602	14.97
Galac.Control No.II	6.20	4.019	4.019		
Inoc.Galactose No.II	9.00	4.019	3.015	1.004	24.97
Galac.Control No.III	6.21	4.019	4.019		
Inoc.Galactose No.III	9.15	4.019	2.913	1.106	27.49

Table 3 - Results of Titration for Third Series of Sugars

Material Titrated	C. C. Reagent	Mg.Sugar per c.c. before Inoc.	Mg.Sugar per c.c. after Inoc.	Mg.Sugar per c.c. Used	Per Cent Sugar Used
Arabinose Control No.I	5.85	4.103	4.103		
Inoc.Arabinose No.I	6.05	4.103	3.765	0.339	8.19
Arab.Control No.II	5.83	4.103	4.103		
Inoc.Arabinose No.II	6.00	4.103	3.793	0.310	7.55
Arab.Control No.III	5.84	4.103	4.103		
Inoc.Arabinose No.III	6.32	4.103	3.679	0.424	10.33
Dextrose Control No.I	5.20	3.792	3.792		
Inoc.Dextrose No.I	7.23	3.792	3.182	0.610	16.08
Dextrose Control No.II	5.23	3.792	3.792		
Inoc.Dextrose No.II	8.45	3.792	2.815	0.977	25.76
Dextrose Control No.III	5.20	3.792	3.792		
Inoc.Dextrose No.III	8.90	3.792	2.678	1.114	29.37
Galac.Control No.I	5.40	4.337	4.337		
Inoc.Galactose No.I	12.00	4.337	1.893	2.444	56.35
Galac.Control No.II	5.45	4.337	4.337		
Inoc.Galactose No.II	11.70	4.337	2.138	2.199	50.70
Galac.Control No.III	5.40	4.337	4.337		
Inoc.Galactose No.III	10.05	4.337	2.513	1.823	42.07

Table 4 - Summarizing Data of Preceding Tables

Sugar	Highest Am't Used(in Mg.)	Lowest Am't Used(in Mg.)	Average Used in Mg.	Average Per Cent
Arabinose	0.424	0.010	0.217	5.275
Dextrose	1.114	0.213	.663	17.095
Galactose	2.444	0.602	1.523	35.660

columns two and three, adding them together, and dividing by two.

Further work was carried on to try to detect the presence of lactic acid, mannitol, acetic acid, ethyl alcohol, acetone, and formic acid.

Five c.c. were withdrawn from the original inoculated media and tested for lactic acid by means of Uffelman's[6] solution. This solution contains 20 c.c. of 4% phenol to which has been added two drops of 2% ferric chloride. The mixture is a deep amethyst color, due to the probable reduction of iron and the formation of phenol ferric chloride. When a few drops of this solution are added to an unknown, the presence of lactic acid is indicated by the appearance of a yellow or greenish-yellow color. The test is sensitive for lactic acid in dilution of 1:10,000. Positive lactic acid tests were obtained for sucrose, galactose, and glucose, but not for lactose nor arabinose. The controls were negative in each case. This entire procedure was repeated and identical results were obtained.

In testing for mannitol[8], 5 c.c. of the inoculated solution were taken and dissolved in 18 c.c. of concentrated HCl, then

shaken with 2 c.c. of benzaldehyde. The presence of mannitol would be indicated by a crystalline precipitate of mannitetribenzacetol (m.p.207° C.). In all cases the results were negative.

For acetic acid[8] the solution was neutralized with .1 N NaOH. The material was then evaporated to dryness, 0.5 gm. of the salts dissolved in 1 c.c. of concentrated H_2SO_4, then warmed over a small flame. If acetic acid is present, the characteristic odor should be produced. For further confirmation, .2 c.c. of ethyl alcohol were added to the concentrated acid solution, and again heated until vapors came off freely. After this the solution was poured into a watch glass containing 5 c.c. of cold water. If acetic acid were present in sufficient concentration, there would have been an odor of ethyl acetate. The tests were negative in all cases.

To test for ethyl alcohol and acetone[8], two drops of a 10% NaOH solution were added per c.c. of the inoculated media. Then, drop by drop, a concentrated solution of iodine (made by dissolving one part I_2 to five parts of KI in fifteen parts of cold H_2O) was added until just a trace of yellow appeared. The material was then allowed to stand at room temperature for two minutes. Afterward the solution was heated to 60° C. for one minute. If ethyl alcohol or acetone were present in concentrations of 1:20 at the lowest, a pre-

cipitate of iodoform would have settled out. No alcohol or acetone were indicated by these tests.

In order to test for formic acid[8], one gram of powdered HgO was added to 5 c.c. of the inoculated solution. The mixture was warmed to 50° C. and then shaken vigorously for one minute. The presence of formic acid is indicated by a gray precipitate of pure mercury according to the following reaction,

$$HgO + CH_2O_2 \longrightarrow Hg\downarrow + CO_2 + H_2O$$

Formic acid may be detected in a 1% concentration when the above procedure is followed. Results were negative.

Summary and Conclusions

1. Experimental evidence indicates that Alcaligines fecalis utilizes dextrose, galactose, and to a much lesser extent arabinose.
2. There was indication of sucrose utilization as shown by a slight turbidity and the production of lactic acid.
3. There was no indication of lactose utilization as shown by the fact that the medium was as clear as the control, and there was no production of lactic acid.
4. The organisms remained viable in plain Munter's solution but there was no indication of growth.
5. When the organisms were inoculated into litmus sugar broths there was no detectable production of acid or gas. In the case of the acid this condition is due probably to the

inability of the litmus to show colorimetrically any slight amount of lactic that may be produced and yet be demonstrable with Uffelman's solution.

6. There were no determinable amounts of mannitol, acetic acid, ethyl alcohol, acetone, or formic acid produced.
7. Lactic acid appeared to be produced in demonstrable amounts.

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