Observations on *Alcaligenes lipolyticus*[1]

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DURING the plating of a large number of samples of milk, cream, butter and various other materials for lipolytic organisms by the use of the nile blue sulfate technic (6) an organism was occasionally encountered which differed from the usual types found in that the colonies were very small in size. An outstanding character of the organism was its pronounced lipolytic ability as evidenced by the rapid hydrolysis of the fat globules under the colonies and by the extent of the zone of hydrolysis.

Evans (3) in 1916 made a study of the bacteria of milk freshly drawn from the normal udder and isolated a species which she designated *Bacillus abortus* var. *lipolyticus*. Evans found that the organism produced a very scanty growth in the form of small separate colonies on meat-infusion agar slants. Gelatin was not liquefied, nitrates were not reduced and acid was not produced from carbohydrates. Growth was slight in litmus skim milk, and the medium remained unchanged; in litmus whole milk there was good growth with slow acid development, which was first apparent in the cream layer. Thirty-seven degrees Centigrade was considered to be the optimum growth temperature.

Later Evans (4) pointed out that *Bacillus abortus* var. *lipolyticus* could be found in large numbers in milk and that it was killed by a temperature of 52° C. for 30 minutes or 63° C. for 30 seconds. The same author (5) in 1918 noted that this organism did not form endospores and accordingly belonged in the genus Bacterium. Since the variety

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designation was unwieldy and the organism was likely a distinct species, she suggested the name *Bacterium lipolyticus*. The organism was found to be nonpathogenic for guinea pigs.

Steck (7) in 1921 studied the bacteria present in the normal udder and reported the occurrence of the type described by Evans. Dorner (2) investigated the bacterial flora of aseptically drawn milk and found a large number of rods which he considered identical with the species isolated by Evans.

SOURCES OF CULTURES

Twenty-one cultures of the organism were isolated. Fifteen were obtained at various intervals from the raw milk of one producer supplying Iowa State College, and one came from the raw milk of another producer. Four cultures were isolated from raw mixed milk, and one was obtained from the water of a lake on the Iowa State College campus.

IDENTITY OF THE ORGANISM

A study of the morphology, cultural characters and biochemical features of the 21 cultures isolated indicated that they were identical with the organism described by Evans (3). This study has permitted an extension of the original description. The characters of the species are such that it probably belongs in the genus Alcaligenes, and the name *Alcaligenes lipolyticus* is suggested.

SPECIAL CHARACTER OF *ALCALIGENES LIPOLYTICUS*

HYDROLYSIS OF FAT

All of the 21 cultures hydrolyzed fat when tested by the nile blue sulfate technic (6) using cottonseed oil and beef-infusion agar, and they were relatively consistent in the type of lipolysis produced under spot colonies. Complete lipolysis beneath the growth occurred with all of the cultures, while all except one hydrolyzed the fat for a considerable distance beyond the edge of the colony.

HYDROLYSIS OF SIMPLE TRIGLYCERIDES

The 21 cultures were tested for their ability to hydrolyze various simple triglycerides dispersed in beef-infusion agar containing nile blue sulfate. All of them hydrolyzed triisovalerin, tricaproin, tricaprylin, tricaprin, trilaurin and triolein. Tripropionin and tributyrin were hydrolyzed by a majority of the cultures; those not bringing about hydrolysis were unable to grow on the media. Trivalerin, triheptylin, trimyristin, tripalmitin and tristearin were not hydrolyzed.

ACTION IN CREAM

Each of the 21 cultures was inoculated into a small amount of sterile cream. After seven days at 21° C. all of the cultures had developed pronounced rancidity.

ACTION IN BUTTER

Each of 11 cultures was inoculated into cream that had been pasteurized at 82° C. for 15 minutes. The cream was then churned and the unsalted butter stored at 21° C. After three days seven of the samples had developed rancidity, while after five days all of the samples were rancid.

PRODUCTION OF ACETYLMETHYLCARBINOL PLUS DIACETYL IN SKIM MILK

Each of four cultures was inoculated into skim milk and the milk incubated at 21° C. for three days. When 200-gm. samples of the cultures were steam distilled in the presence of ferric chloride and the distillates collected in a solution of hydroxylamine hydrochloride and sodium acetate, no nickel dimethylglyoximate was obtained when nickel chloride was added.

Each of two cultures was inoculated into three portions of skim milk to which had been added 0.4, 0.5 and 0.6 percent citric acid, respectively. After three days incubation at 21° C. no acetylmethylcarbinol plus diacetyl was found when the cultures were examined.

PRODUCTION OF VOLATILE ACID

The production of volatile acid was studied with three cultures by inoculating each of them into skim milk and incubating the milk six days at 21° C. When volatile acidity determinations were then made by steam distilling 250-gm. portions of the cultures after the addition of 15 ml. of approximately n/1 H_2SO_4, it was found that no increase had occurred.

EFFECT OF GLYCEROL AND THE SODIUM SALTS OF FATTY ACIDS ON THE GROWTH OF *ALC. LIPOLYTICUS*

The fact that the group of otherwise relatively inert organisms attacked fat so readily suggested that they must use one or more of the products of hydrolysis. This theory was tested by noting the effect of glycerol and of the sodium salts of various fatty acids upon the growth of the organisms. Beef-extract broth and beef-infusion agar were used as controls; glycerol and the sodium salts in various concentrations were added to other lots of broth and agar; and the reactions of the media were adjusted to pH 6.8. Each medium to be tested was inoculated with each of the 21 cultures. The results obtained after an incubation of approximately one week at 21° C. were as follows:

Glycerol. Concentrations of 0.5 percent of glycerol in the broth and agar did not influence the growth of the organisms.

Sodium Acetate. In broth containing 0.5 percent sodium acetate growth was much heavier than in the control tubes. Concentrations between 0.25 and 0.5 percent in agar slants also increased the growth. In the control slopes the growth was thin, dull, beaded and almost streptococcus-like; while in the slopes containing sodium acetate it was luxuriant, white and spreading.

Sodium Propionate. Concentrations of 0.5 percent of sodium propionate in broth greatly aided growth, while the same concentrations in agar completely inhibited development. When 0.25 percent sodium propionate was added to the agar there was good growth, but the growth was not quite as extensive as in the agar to which sodium acetate had been added.

Sodium Butyrate. The addition of 0.5 percent sodium butyrate to broth gave the best growth in the series, and with some cultures pellicle formation was noted. A 0.25 percent concentration in agar gave very good growth as compared with that in the control, while 0.5 percent inhibited growth.

Sodium Caproate. When 0.25 percent sodium caproate was added to broth, growth was greatly stimulated. The same concentration in beef-infusion agar inhibited development, while 0.1 percent gave remarkably good growth.

Sodium Caprylate. Sodium caprylate appeared to be extremely toxic, and the concentrations used were necessarily very low; 0.1 percent in broth and 0.05 percent in agar brought about much better development than that in the control tubes.

Sodium Caprate. Sodium caprate was relatively toxic, as compared with the other compounds used. Concentrations of 0.1 percent in broth and 0.05 percent in agar stimulated growth.

Sodium Oleate. Growth of all the cultures was distinctly aided by the addition of 0.5 percent sodium oleate to broth and 0.25 percent to the agar.

ABILITY TO USE VARIOUS FAT COMPONENTS AS THE SOLE SOURCE OF CARBON

The ability of *Alc. lipolyticus* to use glycerol or certain of the fatty acids as the sole source of carbon was investigated. The synthetic medium A of Ayres, Rupp and Johnson (1) was used as a control. It had the following composition:

Sodium ammonium phosphate	2.0 grams
Dextrose	10.0 grams
Potassium chloride	0.1 grams
Distilled water	1,000.0 ml.

The test media were made up by varying the source of carbon in the above medium. Instead of dextrose, the following compounds were used in the amounts designated: 0.25 percent glycerol, 0.25 percent sodium acetate, 0.25 percent sodium propionate, 0.25 percent sodium butyrate, 0.05 percent sodium caproate, 0.05 percent sodium caprylate, 0.05 percent sodium caprate and 0.1 percent sodium oleate.

All of the cultures were able to grow to a slight extent in the control medium containing dextrose as the sole source of carbon. The organisms were also able to utilize glycerol and the sodium salts of the fatty acids

designated as carbon sources, but the various salts differed in their ability to support growth. The development in the media containing sodium acetate, sodium butyrate and sodium oleate was very good as compared with that in the control tubes; while growth in the media containing glycerol, sodium propionate, sodium caproate, sodium caprylate and sodium caprate was relatively poor.

GENERAL DESCRIPTION OF *ALCALIGENES LIPOLYTICUS*

MORPHOLOGY (CULTURES GROWN AT 21°C.)

Form and Size. Rods; 0.6 to 1.0 by 1.0 to 1.4 microns after one day on beef-infusion agar; after approximately three weeks cells elongated measuring up to 0.8 microns in width and 2.4 microns in length.

Arrangement. Singly and in pairs.

Motility. Non-motile.

Staining Reactions. Generally gram positive in young cultures. Older cultures gram negative.

Spores. None observed in either young or old agar cultures.

CULTURAL CHARACTERISTICS (CULTURES GROWN AT 21°C.)

Agar Slope. Scanty, white, filiform, nonviscid, dull growth on beef-infusion agar after 1 to 2 days, the type of growth not changing on extended incubation.

Agar Stab. Small amount of surface growth on beef-infusion agar with growth extending downward along the line of inoculation.

Agar Colony. Growth evident on beef-infusion agar after approximately 2 days; after 4 days surface colonies white, nonviscid, round with entire edge and from 1 to 2 mm. in diameter. Subsurface colonies oval, white, nonviscid and smaller than surface colonies.

Gelatin Stab. No liquefaction. Scanty growth on surface with some growth following the line of inoculation.

Bouillon. A slight cloudiness in the medium and a slight sedimentation after 3 to 4 days.

Potato. No visible growth.

Litmus Skim Milk. Beyond a slight precipitate in the tube on extended incubation, no visible action in litmus skim milk.

Litmus Whole Milk. Acid apparent in the cream layer after approximately 2 weeks, the acid later extending down through the milk.

BIOCHEMICAL FEATURES (CULTURES GROWN AT 21°C.)

Indol. Not produced.

Nitrates. Not reduced.

Hydrogen Sulphide. Not produced in agar.

Methyl Red Reaction. Negative.

Voges Proskauer Reaction. Negative.

Fermenting Power. Most of the cultures produced neither acid nor gas from the compounds used. Acid but no gas produced from arabinose, dextrose, lactose, levulose and maltose by a few cultures. Galactose, glycerol, inulin, mannitol, raffinose, salacin and sucrose not fermented and starch not hydrolyzed.

Hydrolysis of Fat. Fat hydrolyzed.

Hemolysis. Red cells not hemolyzed.

GROWTH CONDITIONS

Oxygen Relationships. Aerobic.

Growth Temperatures. Growth by all cultures at 10° C., 37° C., and at temperatures in between. At 40° C. slight growth by most of the cultures.

SUMMARY

A number of lipolytic cultures were isolated and were considered identical with *Bacillus abortus* var. *lipolyticus* described by Evans (3). The characters of the organism indicate that it belongs in the genus Alcaligenes; therefore, the name *Alcaligenes lipolyticus* is proposed. *Alc. lipolyticus* produced rancidity in butter and was characterized by its ability to rapidly hydrolyze fat and to use certain of the salts of fatty acids as the sole source of carbon. A description of *Alc. lipolyticus* is presented.

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