

Characteristics of Sporulating Facultative Bacteria Producing Gas from Lactose.

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Facultative spore-forming bacteria, fermenting lactose with gas production, have been encountered on a number of occasions in water supplies. As these bacteria are gram negative and frequently do not show spores on carbohydrate media, they have been a source of confusion in the interpretation of bacterial water analysis for the incidence of members of the colon group. Such organisms, which were isolated for the first time in Iowa from the chlorinated drinking water at Iowa City in 1921 by Hinman and Levine, were thought to be rare; but evidence is accumulating to indicate that they are quite ubiquitous, having since been isolated from soil, animal and human feces, decaying and canned vegetables, sewage, grains, and eggs.

There is considerable confusion in the literature concerning the identity and differentiation of species allocated to this group of bacteria. This report presents, briefly, a study of all strains described in the literature, which are available, together with a number of freshly isolated cultures.

The study included 63 freshly isolated strains and authentic cultures of all described strains except 2 which apparently are extinct.

There was considerable variation in the size of the bacteria on different culture media. In a few cases terminal oval spores were seen within the sporangia, but generally they were observed free. All strains were actively motile.

Culturally, the strains showed very little difference. On nutrient agar at 37°C., growth was slight, effuse, and transparent. On sugar-containing agar, growth was spreading and slimy. Growth in nutrient broth was slight and with some sediment; in sugar broth the medium frequently became very viscous.

Colony characteristics vary with the medium. On nutrient agar, well isolated colonies are small and transparent. On sugar-containing media, colonies are extremely variable, but are transparent and usually very slimy. On Endo's medium, colonies are pink to red in color and may be small and round or large and amoeboid. Development of a sheen and red halo around the colonies were not constant characteristics. On eosin methylene blue agar, growth

was markedly retarded; small pin-head colonies appeared after 48 to 72 hours; the production of a sheen was variable.

On the basis of the physiological tests employed, the organisms fall into 2 groups (Table I) which may be differentiated into a (V.P.—) group, which grew well at 45°C. and fermented sorbitol and rhamnose with acid and gas production; and a (V.P.+) group which did not grow at 45°C. and did not produce acid or gas from sorbitol and rhamnose. These subdivisions will be referred to as the macerans (V.P.—) and the polymyxa (V.P.+) groups, respectively.

TABLE I.
Differential Characteristics in Facultative, Sporulating, Aerogenic Bacilli.

No. of Strains Character	<i>Aero. macerans</i> group	<i>Aero. polymyxa</i> group
	16 % Positive Reactions	71 % Positive Reactions
Growth at 45°C. (48 hr.)	100	0
Growth at 13°C. (1 week)	0	100
Acid and gas in Sorbitol (72 hr. 37°C.)	100	0
Acid and gas in Rhamnose (48 hr. 37°C.)	100	0
Voges-Proskauer reaction (72 hr. 37°C.)	0	100
Gelatin liquefaction (96 hr. 37°C.)	0	88.8
Milk coagulated (72 hr. 37°C.)	0	84.5
Agglutinated by <i>Aero. macerans</i> serum (2 sera tested)	100	0
Agglutinated by <i>Aero. polymyxa</i> serum (9 sera tested)	0	*

* The 9 sera show that the group is very heterogeneous serologically. Each tested serum agglutinated its specific organism and a number of other strains, but no one serum agglutinated the entire group.

Indol and H₂S were not produced; citric and malonic acids were not utilized as a sole source of carbon by any of the strains; nitrates were reduced to nitrites by all cultures.

The organisms were tested in 33 carbohydrates and polyatomic alcohols. Most of these were fermented with acid and gas production; however, only rhamnose and sorbitol seemed to be of any differential value.

Serologically, the 2 groups appear to be distinctly different. The macerans group is an antigenic entity; the polymyxa group, however, shows a great deal of heterogeneity and further work is in progress. The strains tested were not pathogenic for rabbits.

The results indicate that there exist 2 distinct groups among the facultative, sporulating aerogenic bacilli. The 2 groups are strikingly different, physiologically and serologically.

If the genus *Aerobacillus* Donker (1926) is to be adopted, the following species are recognized for the present:

1. *Aerobacillus polymyxa* (Prazmowski) Donker 1926, syn:
 - Clostridium polymyxa* Prazmowski 1880
 - Granulobacter polymyxa* Beijerinck 1893
 - Bacillus polymyxa* Beijerinck and Den Dooren De Jong 1923
 - Astasia asterospora* Meyer 1892
 - Bacillus asterosporus* (Meyer) Migula 1900
 - Bacillus mycoides* var. *ovoaethylicus* Wagner 1916
 - Bacillus aerosporus* Greer 1928.
2. *Aerobacillus macerans* (Schardinger) Donker 1926, syn:
 - Bacillus macerans* Schardinger 1905
 - Bacillus acetoethylicum* Northrop 1919
 - Aerobacillus acetoethylicus* (Northrop) Donker 1926.

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Ligation of Carotid and Vertebral Arteries in Monkeys.

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The mortality resulting from ligation of the common carotid artery in man varies from 7 to 90%, depending on the condition for which the ligation was done, and the age of the patient.¹ The value of pre-ligation compression of the carotid artery to establish collateral circulation, is not definitely established. This work was primarily undertaken to determine the effect of pre-ligation compression of the common carotid in monkeys. Dogs and rabbits seldom develop symptoms after ligation of a common carotid artery,² whereas we hoped that monkeys, whose cranial blood supply is quite similar to that of man,³ would react in a way comparable to man. However, we were unable to cause paralysis or death in monkeys by ligation of a common carotid artery, or by simultaneous unilateral ligation of vertebral, internal, external and common carotid arteries. The ligations were continued in an effort to determine

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¹ Freeman, L., *Ann. Surg.*, 1921, **74**, 316.

² Sussi, L., *Ann. ital di chir.*, 1932, **11**, 311.

³ Hartman and Straus, *Anatomy of the Rhesus Monkey*, Chapter XII, p. 251.