THE FACULTATIVE SPORULATING BACTERIA PRODUCING GAS FROM LACTOSE

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Facultative spore-bearing bacilli capable of fermenting carbohydrates with the formation of gas in the Durham tube have been reported by many investigators. Such organisms, once thought to be rare, appear to be ubiquitous for they have been isolated from water, soil, human and animal feces, sewage, eggs, decaying and canned vegetables, and grains. The bacteria of this group have attracted attention because of their ability to produce considerable quantities of acetone and ethyl alcohol from carbohydrates, and also on account of their occasional occurrence in water supplies. Since they are Gram-negative and usually do not form spores on carbohydrate media, they have been a source of confusion in the interpretation of bacterial water analysis for the incidence of the colon group.

There is some confusion in the literature concerning the identity and differentiation of members of this group. This report presents briefly a study of all available strains described in the literature, together with a number of freshly isolated cultures.

Donker (1926) proposed that a new genus be created to include the facultative sporulating bacteria that are motile by means of peritrichous flagella, form clostridia, produce catalase and ferment carbohydrates with the production of gas. He suggested the generic name *Aerobacillus* for these organisms which he considered as having some of the characteristics of the genera *Aerobacter* and *Bacillus*. However, Pribram (1929 and 1933) employed the generic name *Aerobacillus* to include only the aerobic bacteria which are motile by means of peritrichous flagella, bear terminal spores, and are Gram-negative.

From the description of the organisms listed in this genus by Pribram, it is evident that they do not conform to the characterization of the genus *Aerobacillus* of Donker. On the other hand, such organisms as *Bacillus asterosporus* and *Bacillus polymyxa*, listed by Donker in the genus *Aerobacillus*, are allocated by Pribram to his genus *Bacillus*. It is apparent, therefore, that the generic designation *Aerobacillus* as employed by Donker and by Pribram does not refer to the same group of organisms. Due to priority of Donker, his designation should be adopted if the genus *Aerobacillus* is found to be desirable. It is in this latter sense that the term will be employed in this contribution. (Bergey (1934) includes these species in the genus *Bacillus*.)

Five species of the genus were described by Donker, namely:

1. *Aerobacillus polymyxa* (Prazmowski) Donker
   Syn: *Clostridium polymyxa* Prazmowski
   Granulobacter polymyxa Beijerinck
   Bacillus polymyxa Beijerinck and den Dooren de Jong
   Bacillus asterosporus (A. Meyer) Migula
2. *Aerobacillus acetoethylicus* (Northrop) Donker
   Syn: *Bacillus acetoethylicum* Northrop
3. *Aerobacillus macerans* (Schardinger) Donker
   Syn: *Bacillus macerans* Schardinger
4. *Aerobacillus violaris* (Bréaudat) Donker
   Syn: *Bacillus violarius acetonicus* Bréaudat
5. *Aerobacillus amaracrylus* (Voisenet) Donker
   Syn: *Bacillus amaracrylus* Voisenet

A brief review of the literature concerning each organism listed above, is presented to facilitate a proper understanding of this group of bacteria.

1. AEROBACILLUS POLYMYXA

Prazmowski (1880) described an organism that closely resembled *Clostridium butyricum* but could grow in the presence of air. Starch and cellulose were strongly attacked, and carbon dioxide gas was formed from some carbohydrates. The organism was designated as *Clostridium polymyxa*. Beijerinck (1893 and 1896) studied an organism that grew best aerobically and

produced slime in carbohydrate media. Gases were formed, and upon analysis were found to consist of carbon dioxide and small amounts of hydrogen. The organism was described as rod-shaped and motile, and produced spores and granulose. Its discoverer placed it in the genus *Granulobacter* (*Granulobacter polymyxa*) and reported it to be found "normally on grains of wheat and very plentiful in garden soil." In later studies of this organism, Beijerinck and van Delden (1902) reported cultural differences which led them to recognize two varieties, namely, *Granulobacter polymyxa* var. *mucosum* and *Granulobacter polymyxa* var. *tenax*.

Gruber (1905) isolated an organism from milk which he thought was identical with *Clostridium polymyxa*. Growth was best in the absence of air but spores were formed only under aerobic conditions.

Meyer (1892) isolated from carrots an organism to which he gave the name *Astasia asterospora* because of its ridged spore resembling a star. The organism was unusual also in that it presented a new type of arrangement of flagella, and that a nucleus was observed in the cells. These peculiarities attracted the attention of Migula (1898) who obtained a culture for study, but was unable to confirm the observations of Meyer. Subsequently, Meyer (1899) acknowledged his previous error and confirmed the work of Migula. Aderhold (1899) observed *Astasia asterospora* in canned asparagus.

Migula (1900) included *Astasia asterospora* in the genus *Bacillus*, listing it as *Bacillus asterosporus* (Meyer) Migula. In 1901, Gottheil suggested that probably *Bacillus subanaerobius* Gruber (1887) and *Bacillus thalassophilus* Russell (1892) were synonymous with *B. asterosporus* (Meyer) Migula.

Behrens (1902, 1903) observed that *B. asterosporus* in pure culture would ret flax and hemp. Chester (1903) studied a culture of *B. asterosporus*, supposedly isolated by Meyer, and gave a fairly complete description of its characteristics. In 1903 Meyer again published results of his researches on this organism. It is interesting that he reported, contrary to other workers, that most of the gas produced by this culture was hydrogen

rather than carbon dioxide. Wund (1906) observed that an atmosphere containing 100 mgm. of oxygen per liter was optimum for spore formation of *B. asterosporus*. This work was later confirmed by Meyer (1909).

Bleau (1905) reported the optimum temperature for *B. asterosporus* to be about 35°C., with spore formation largely inhibited at 40° to 45°C.

B. asterosporus was found in the intestinal canal of cattle by Ankersmit (1906) and in decaying vegetables by Wahl (1906). Hasselhoff and Bredemann (1906) isolated a number of organisms from vegetables and named three new species, *Bacillus clostridioides*, *Bacillus dilaboides* and *Bacillus asterosporus* (*alpha*). In a later publication Bredemann (1909b) concluded that these forms were sufficiently alike to be considered as one species, *B. asterosporus*.

Other workers who have reported researches concerning *B. asterosporus*, but whose contributions space does not allow us to consider here, are Garbowski (1907), Ritter (1908), Bredemann (1909c), Meyer (1909), Eisenberg (1909), Viehover (1912), Barthel (1922), Lisk (1923), Virtanen and Karström (1925), McFall (1929), Stapp and Zycha (1931), Patrick (1931) and Zycha (1932).

2. AEROBACILLUS ACETOETHYLICUS

In an attempt to find a cheap method for the production of acetone, Northrop, Ashe and Senior (1919) isolated from decaying potatoes an organism which they named *Bacillus acetoethylicum*, since its most striking characteristic was the formation of acetone and ethyl alcohol. The organism was described in detail as a motile sporulating rod, and Gram-negative, a facultative anaerobe, with extensive fermentative powers. Solutions of molasses served as a satisfactory substrate for the organism, and fairly large quantities of acetone and ethyl alcohol were obtained when the reaction was adjusted to pH 8.5 to 9.5 (Northrop, Ashe and Morgan (1919)).

Peterson and Fred (1920), studying intermediate products of the fermentation of carbohydrates, noted that acetaldehyde was

produced by the acetone-forming organism, *B. acetoethylicum*. The products of fermentation of various substrates by *B. acetoethylicum* have been investigated by Arzberger, Peterson and Fred (1920); Peterson, Fred and Verhulst (1921); Juritz (1921); Fred, Peterson and Anderson (1923); Speakman (1925); Bakonyi (1926); and Patwardhan (1930).

Donker (1926) recognized *Aerobacillus acetoethylicus* as a separate species, although his description showed that it was closely related to *Aerobacillus polymyxa* and *Aerobacillus macerans*.

3. AEROBACILLUS MACERANS

In 1904 Schardinger described a rod-shaped spore-bearing facultative organism that produced gas and quantities of acetone and ethyl alcohol from carbohydrates. The following year (1905) he published a more detailed description of the organism and gave it the name *Bacillus macerans* (bacillus of retting). Other researches in which he studied the fermentative behavior of this culture were published by Schardinger in 1907, 1909 and 1911. Euler and Svanberg (1922), in a study of the effect of reaction on the growth of *B. macerans* and the course of starch splitting, found the optimum acidity for the growth of the organism in the starch medium to be about pH 6.8.

Hinman and Levine (1922) isolated a number of strains of facultative, spore-forming, lactose fermenting organisms from Iowa surface waters. The morphological and biochemical characteristics of the strains caused the writers to conclude that they were dealing with *B. macerans* or *B. acetoethylicus*.

Coles (1926), in a study of the digestion of pectin and methylated glucoses by various organisms, employed two strains isolated by Hinman and Levine and reported that both fermented pectin with the production of acid and gas. The decomposition of starch by *B. macerans* was studied by Samec (1927). Burkey (1928), in a study of the fermentation of cornstalks and their constituents, isolated two organisms which differed from those isolated by Hinman and Levine only in that they liquefied gelatin.

Zacharov (1930) reported that *B. macerans* produces only

ethyl alcohol, never butyl alcohol, and that the production of acetone and ethyl alcohol is in ratio of one to two.

Meyer (1935) observed that organisms of the *B. macerans* group were present in his crude cultures of cellulose-decomposing organisms.

Donker (1926) listed *B. macerans* as one of the five species of the genus *Aerobacillus*.

4. AEROBACILLUS VIOLARIS

Bréaudat (1906) isolated from polluted water an organism which he described as rod-shaped, Gram-negative (at three days), motile, facultative, spore-bearing, and producing acetone from sucrose. A deep violet pigment was formed on potato and on agar media in the presence of peptone and air. He named the organism *Bacillus violarius-acetonicus*.

The description by Bréaudat is incomplete, and there is a possibility that his organism does not belong with the other species discussed in this paper. A very important characteristic possessed by all species of the genus *Aerobacillus* is the ability to produce gas in the decomposition of carbohydrates. Bréaudat failed to mention whether his organism possessed this characteristic, and the culture is no longer available. Dr. A. R. Prévot, Chief of the Laboratory at the Institute Pasteur, stated in a private communication that this organism was no longer alive.

5. AEROBACILLUS AMARACRYLUS

Voisenet (1911, 1913, 1914, 1918) described an organism which he found in water and also in bitter wines. He studied particularly its ability to dehydrate glycerol with the formation of acrolein, and because of this interesting characteristic he called it *Bacillus amaracrylus* (1913). The organism was described as rod-shaped, spore-bearing, motile and Gram-positive, and produced gas in the fermentation of carbohydrates.

Warcollier and LeMoal (1932) and Warcollier, LeMoal and Tavernier (1934) noted the presence of acrolein in cider and wine and concluded that the responsible organism was similar to *B. amaracrylus* or to *Clostridium welchii*.

Donker (1926) and McFall (1929) included Voisenet's species in the genus *Aerobacillus*. No experimental work was done with the organism by these authors and we have been unable to obtain a culture for the present study.

6. OTHER "AEROBACILLI"

A number of organisms have been described in the literature which seem to possess the essential characteristics of the genus *Aerobacillus* Donker. Only a brief review of these reports is necessary.

Wagner (1916) isolated an organism from eggs that he named *Bacillus mycoides* var. *ovoaethylicus*, and which Perlberger (1924) described as a motile rod, spore-bearing, and producing acid and gas in a number of carbohydrates, polyatomic alcohols and glucosides. Perlberger concluded that the organism was not one of the *Bacillus mycoides* group, but was closely related to *B. asterosporus*. Pribram (1933) stated that the Wagner culture was similar to *B. polymyxa* and designated it *B. ovoaethylicus*.

Greer and his co-workers (1928) in a series of papers dealing with the sanitary significance of lactose-fermenting bacteria not belonging to the *Bacillus coli* group, described organisms that were aerobic, spore-forming and fermented lactose with the production of acid and gas. The name *Bacillus aerosporus* was given to the group. In their work about sixty strains were used, all of which were very similar and differed little from previously described organisms. They were able to isolate *B. aerosporus* from 17 out of 18 samples of horse manure, 11 out of 14 samples of cow dung, 1 out of 18 samples of human feces, 3 out of 44 samples of sewage, and 7 out of 9 samples of fertilized soil.

Coolhaas (1928) isolated and described an organism very similar to that of Schardinger's, except that it was more thermophilic. He called it *Bacillus thermoamylolyticus*.

Bacillus pandora was isolated from *Hevea latex* by Corbet (1929, 1930) and described as spore-forming, facultative, and producing gas in the fermentation of lactose and sucrose.

Bacterium hessii was isolated from slimy milk by Guillebeau (1891) and was reported to produce some gas in milk agar. The

organism was transferred to the genus *Bacillus* by Flügge (1896), and Neide (1904) stated that it was probably synonymous with *Bacillus silvaticus*. The meager description of the original culture does not justify placing it in the group of "aerobacilli," and the organism is no longer available for study.

Meyer (1918), Ewing (1919), Ellms (1920), Weight (1924), Norton and Weight (1924), Ginter (1927), and Koser and Shinn (1927), isolated and described organisms which, because of their ample descriptions, can undoubtedly be considered as belonging to the "aerobacilli." Other writers who have probably noted the occurrence in their studies of aerobic spore-forming bacteria fermenting carbohydrates with gas production, are: Burton and Rettger (1917), Hall and Ellefson (1918 and 1919), Perry and Monfort (1921), Havens and Dehler (1923), Raab (1923), Sohn (1924) Gettrust and Hostetter (1925 and 1930), Berry (1925), and Janzig and Montank (1928).

Zeissler (1930), Hall (1935) and others have observed that certain anaerobic species of bacilli are capable of delicate aerobic growth on agar media. Some of these microaerophilic organisms, such as *Bacillus carnis* and *Clostridium tertium*, resemble the "aerobacilli" in that they ferment carbohydrates with gas production. The species mentioned differ from those in the "aerobacillus" group in that catalase is not produced, and growth is almost completely inhibited by free oxygen, except on media reduced by the addition of blood or some other reducing material. Hall reported that sporulation of the "microaerophilic" anaerobes was inhibited by free oxygen. The aerobacilli, on the other hand, sporulate readily under aerobic conditions. Therefore, it seems evident that the oxygen-tolerant anaerobic species referred to, do not belong in the genus *Aerobacillus* Donker.

SOURCE OF CULTURES

In this study all available cultures having the characteristics of the genus *Aerobacillus* Donker were employed. Some of the acquired cultures failed to conform to previous descriptions which would place them in the group of "aerobacilli." *B. macerans* (Berlin) and *B. asterosporus* 62a (Apia, Samoa), secured from the

Pribram collection, failed to produce gas from any carbohydrates and were therefore not included. Also, the culture of *B. thermoamylolyticus* received from N. L. Söhngen, did not satisfy the requirements of the genus and was not used in this study. Table 1 shows the source, designation and species allocation of the cultures studied. All named species of the genus were available for study except *B. violarius-acetonicus* Bréaudat and *B. amaracrylus* Voisenet, which are apparently no longer in existence.

In addition to the previously described organisms, 63 strains isolated in this laboratory were studied. These strains were obtained from various sources and purified by the serial dilution pour plate method. Because of the slimy nature of most of the organisms, each strain was replated at least twenty times.

MORPHOLOGY

Smears prepared from glucose agar and nutrient agar cultures after incubation for 18 hours, 36 hours, 3 days and 7 days, were stained with methylene blue and by Gram's method (Hucker modification). Cell measurements were made with a Filar micrometer. All cultures studied were rod-shaped and varied from approximately 2.5 to 6.0 microns in length and from 0.6 to 1.1 microns in width. On media containing fermentable sugar the cells were somewhat larger than on sugar-free media. The endospores were elliptical, about 0.8 by 1.4 microns, and when seen in the cells were located terminally or subterminally. Spores were not observed in glucose agar cultures. All strains were motile when hanging drop examinations were made of 16-to-18 hour (37°C.) nutrient broth cultures. All cultures were Gram-negative at 18 hours (37°C.).

CULTURAL CHARACTERISTICS

Plain nutrient agar. Moderate, spreading, effuse transparent growth; medium unchanged; no distinct odor and no chromogenesis.

Glucose agar. Some strains produced growth comparable to that on plain nutrient agar, while other strains grew abundantly and produced raised slimy colonies.

TABLE 1

Source, designation and species allocation of faculative, sporulating, aerogenic bacilli

SOURCE	NUMBER OF STRAINS	ORIGINAL NAME	ISOLATED BY	RECEIVED FROM	SPECIES ALLOCATION
Psyllium seed	1		McCleskey (1931)		
Grains	5		Porter (1933)		
Sewage	1		Porter (1933)		*Aerobacillus macerans*
Lake water	1		Porter (1933)		
Potato	2	*B. acetoethylicum*	Northrop, et al. (1917)	Amer. T. C. C.	
Potato	1	*B. acetoethylicum*	Northrop, et al. (1917)	J. H. Northrop	
Potato	1	*B. macerans*	Schardinger (1904)*	Amer. T. C. C.	
Potato	1	*B. macerans*	Schardinger (1904)*	Pribram, Vienna	
Water	2	*B. macerans*	Hinman-Levine (1921)	I. S. C. Lab.	
Water	1			O. K. Stark	
Psyllium seed	21		McCleskey (1931)		
Canned rhubarb	1		McCleskey (1934)		
Soil	15		McCleskey, Porter (1933)		
Grains	2		Porter (1933)		
Soil	7	*Aerobacillus polymyxa*	McFall (1928)	I. S. C. Lab.	
Corncobs	2	*Aerobacillus polymyxa*	McFall (1928)	I. S. C. Lab.	
Spinach	1	*Aerobacillus polymyxa*	McFall (1928)	I. S. C. Lab.	*Aerobacillus polymyxa*
Cauliflower	1	*Aerobacillus polymyxa*	McFall (1928)	I. S. C. Lab.	
Beets	1	*Aerobacillus polymyxa*	McFall (1928)	I. S. C. Lab.	
Potato	4	*Aerobacillus polymyxa*	McFall (1928)	I. S. C. Lab.	

Endive, malt, lettuce	4	*Aerobacillus polymyxa*	Donker (1926)*	Amer. T. C. C.	
Water	2			Amer. T. C. C.	
Soil	1		Weight (1926)	Univ. Utah	
Human feces	1		Weight (1926)	Univ. Utah	
Water	1		Ginter (1925)	Univ. Utah	*Aerobacillus polymyxa*
Eggs	1	*B. mycoides* var. *ovoaethylicus*	Wagner (1916)*	Pribram, Vienna	
Soil	1	*B. asterosporus*	Bredemann (1909)*	Pribram, Vienna	
Carrots	1	*Astasia asterospora*	A. Meyer (1892)*	Pribram, Vienna	
Water	1	*B. aerosporus*	Greer, et al. (1928)*	Chicago Bd. H. Lab.	
Water	2		Kauffmann (1934)*	J. Smit, Amsterdam	
Soil	1	*B. asterosporus*	Bredemann (1909)*	Univ. Marburg	

* Date of publication.

Broth. Slight clouding and very little sediment. In sugar broth, some of the cultures produced slime.

Gelatin. Scanty to moderate filiform growth along the line of puncture (20°C.). Strains which liquefied gelatin produced crateriform liquefaction.

Potato. Many strains were able, in 48 hours at 37°C., to reduce potato to a soft pulp. Some strains however lacked this strong diastatic action. A distinct fruity odor was produced. The color of the growth on potato varied from white to light tan.

Loeffler's blood serum. Scanty, effuse growth, with no change in the medium.

Colony characteristics. On plain agar, surface colonies were irregular in form, usually smooth, effuse, and transparent, with no distinct internal structure. Subsurface colonies were circular or elliptical, with entire edge, and granular internal structure. On sugar agar with china-blue indicator there was considerable strain variation in type of colony. Some strains produced round convex slimy colonies, while others produced colonies which were flat and amoeboid. All colonies produced acid. The colonies produced on Endo's agar were similar to those described for china-blue agar. On eosine methylene-blue agar, however, growth of all strains was almost prevented; pinhead colonies with distinct metallic sheen were present after 48 hours at 37°C. Growth on blood agar was abundant with only two strains producing slight hemolysis after 24 hours at 37°C. Colony characteristics were not correlated with the physiological differences noted below.

PHYSIOLOGICAL CHARACTERISTICS

Temperature relations. Sucrose broth, in Durham fermentation tubes, and nutrient broth were incubated at 13°, 20°, 30°, 37°, 42°, 45°, and 50°C., until the medium reached a constant temperature. The tubes were then inoculated and replaced in the incubators at the indicated temperatures. The results of the experiment are given in table 2. On the basis of temperature requirement for growth, the cultures fall into two groups. Those which grew well at 42° to 45°C., but poorly if at all at 20°C., will

be designated as the "*macerans*" group; those that grew luxuriantly at 20°C., but slowly if at all at 42° to 45°C., will be referred to as the "*polymyxa*" group.

Oxygen relationship. For anaerobic studies, McIntosh and Fildes jars were employed. The cultures grew on plain nutrient agar slants under either aerobic and anaerobic conditions, hence they are considered as aerobic and facultative. The "*macerans*" group grew somewhat more luxuriantly under anaerobic conditions than did the "*polymyxa*" group.

Acetyl methyl carbinol production was determined in Bacto M.R.-V.P. medium after incubation for 3 days at 37°C. The indicator employed was the O'Meara reagent as modified by Levine, Epstein and Vaughn (1934). The cultures fall into two groups based on the production of acetyl methyl carbinol. The

TABLE 2

Temperature relations of facultative, sporulating, aerogenic bacilli

SPECIES ALLOCATION	PER CENT POSITIVE (SHOWING GROWTH)					
	13°C. 1 week	20°C. 24 hours	37°C. 24 hours	42°C. 24 hours	45°C. 24 hours	50°C. 24 hours
Aerobacillus polymyxa	100	100	100	0	0	0
Aerobacillus macerans	0	0	100	100	100	0

strains of the "*polymyxa*" (low temperature) group produced acetyl methyl carbinol, whereas the "*macerans*" (high temperature) group did not.

Relationship to reaction of medium. All the strains were inoculated into glucose broth, adjusted and buffered at pH values of 3.4, 4.4, 5.6, 6.0, 7.0, 8.0, and 8.5. All of the organisms grew well within 48 hours in all the media except those at pH 3.4 and 4.4. No growth occurred in the latter media after two weeks incubation at 37°C. The upper limits of pH supporting growth were not determined.

Indol production was determined in Bacto tryptophane broth after incubation at 37°C. for 3, 5 and 7 days. Kovac's reagent was employed as the indicator. All strains were negative.

Production of hydrogen sulphide. To detect hydrogen sulphide

production three media were tried: Bacto Kligler lead acetate agar; the medium of Patrick and Werkman (1933); and the medium proposed by Levine, Vaughn, Epstein and Anderson (1932). None of the cultures produced hydrogen sulphide in the media employed.

Reduction of nitrate to nitrite was determined in a 0.1 per cent peptone solution to which was added 0.02 per cent KNO_3 and 0.05 per cent NaCl. Incubation was at 37°C. for 3 days, and the test reagent employed was sulphanilic acid and naphthylamine-acetate. All the strains reduced nitrate to nitrite. Gas was not produced.

Litmus milk. All of the cultures decolorized the litmus and produced acid and gas. None of the "*macerans*" group coagulated the milk or caused visible peptonization, whereas most of the "*polymyxa*" strains caused both coagulation and partial digestion (table 4).

Utilization of simple triglycerides and natural fats. The method used was that of Collins and Hammer (1934). The substances tried were tri-propionin, butter fat, lard, and cottonseed oil. Incubation was at 20° and 37°C. for three days or longer. None of these materials were utilized.

Utilization of citric and malonic acids. Bacto Koser's citrate medium and Leifson's sodium malonate medium were employed. None of the strains were able to utilize the salts of the organic acids under the conditions of the experiment.

Fermentation reactions. Standard Durham fermentation tubes were employed to determine the ability of the cultures to produce acid and gas in the following substances: *l*-arabinose, *d*-xylose, rhamnose, *d*-glucose, galactose, levulose, *d*-mannose, lactose, maltose, melibiose, sucrose, trehalose, cellobiose, raffinose, melezitose, starch, dextrin, glycogen, inulin, pectin, xylan, aesculin, amygdalin, salicin, saponin, *a*-methyl-glucoside, glycerol, erythritol, adonitol, mannitol, sorbitol and dulcitol.

Of the above compounds, erythritol, adonitol, dulcitol and inositol were not fermented by any cultures. The "*macerans*" (high temperature, V.P. negative) group fermented sorbitol and rhamnose with the production of acid and gas, where the "*poly-*

myxa" (low temperature, V.P. positive) group failed to ferment either. All the other substances were attacked, and acid and gas were produced by all strains of both groups, except three strains of the "*polymyxa*" group which did not ferment glycerol.

SEROLOGY

Pathogenesis. These organisms are considered to be non-pathogenic for rabbits. Two strains of the "*macerans*" group and 9 strains of the "*polymyxa*" group produced no symptoms of disease when living cultures were injected intravenously into

TABLE 3

Serological characteristics of facultative, sporulating, aerogenic bacilli

SERA	ANTIGENS—PER CENT OF STRAINS AGGLUTINATED	
	Aerob. polymyxa (71 strains)	*Aerob. macerans* (16 strains)
Bacillus macerans (Schardinger)	0	100
Bacillus acetoethylicus (Northrop et al.)	0	100
Bacillus asterosporus (Bredemann)	26.76	0
Bacillus asterosporus (Bredemann)	23.94	0
Astasia asterospora (A. Meyer)	49.29	0
Bacillus aerosporus (Greer et al.)	36.62	0
B. mycoides var. *ovaethylicus* (Wagner)	22.53	0
Aerobacillus polymyxa (Donker)	60.56	0
Aerobacillus polymyxa (Donker)	36.62	0
Aerobacillus polymyxa (Authors)	56.33	0
Aerobacillus polymyxa (Authors)	54.92	0

rabbits. Previous investigators have considered them non-pathogenic for mice.

Agglutinin production. Heat-killed saline suspensions of selected strains were injected into the marginal ear vein of healthy rabbits. The cultures employed were selected as being representative of the various types described in the literature and, where possible, the original strains were utilized.

The 11 organisms selected were:

Bacillus macerans, original strain of Schardinger.
Bacillus acetoethylicus, original strain of Northrop *et al.*

Bacillus asterosporus, 2 strains, supposed to be the original strains of Bredemann.
Astasia asterospora, original strain of A. Meyer.
Bacillus aerosporus, original strain of Greer *et al.*
Bacillus mycoides var. ovoaethylicus, original strain of Wagner.
Aerobacillus polymyxa, 4 strains; 2 strains (839 and 840 A.T.C.) studied by Donker, and 2 strains isolated in this laboratory.

Macroscopic agglutination tests were carried out in the usual way with suspensions prepared with 0.4 per cent c.p. NaCl and 0.2 per cent formaldehyde in distilled water. The results are summarized in table 3.

The agglutination reactions served to divide the cultures into two groups. The "*macerans*," or V.P. negative, high temperature group was found to be antigenically homogenous, but the "*polymyxa*," or V.P. positive, low temperature group proved to be serologically heterogeneous. Although nine "*polymyxa*" sera were prepared, a small number of strains were not agglutinated by any of their sera. Each serum agglutinated its homologous organism and a number of others, but no one serum agglutinated more than about 60 per cent of the "*polymyxa*" strains.

SUMMARY

A study of the group of facultative spore-bearing bacteria which ferment carbohydrates with the production of gas has been made for the purpose of determining their systematic relationships. Eighty-seven strains, which had been isolated from such varied sources as decaying and canned vegetables, water, soil, feces, eggs and grains, were employed. Included in this number were all available organisms of this group which have been isolated and reported in the literature, e.g., the original strains of A. Meyer, Bredemann, Wagner, Schardinger and Northrop.

From the results of this study it seems that the facultative, sporulating, aerogenic bacteria fall naturally into two groups: The "*macerans*" group, of which *Bacillus macerans* Schardinger is typical; and the "*polymyxa*" group, of which the organism isolated by Meyer (1892) and named *Astasia asterospora* is the

oldest representative extant. The reactions which differentiate the "*macerans*" and "*polymyxa*" groups are indicated in table 4.

From the results obtained in this study it seems that there are two distinct species in the group of facultative sporulating aerogenic bacteria, and if the genus *Aerobacillus* Donker is to be adopted, the specific names *Aerobacillus polymyxa* and *Aerobacillus macerans* are suggested.

The genus *Aerobacillus* should include the spore-forming rods which grow aerobically and anaerobically, produce catalase, and decompose carbohydrates with the production of acid and gas

TABLE 4

Differential characteristics in facultative, sporulating, aerogenic bacilli

CHARACTER	PER CENT POSITIVE REACTIONS	
	Aerob. macerans group (16 strains)	*Aerob. polymyxa* group (71 strains
Growth at 42–45°C. (48 hours)	100	0
Growth at 13–20°C. (1 week)	0	100
Acid and Gas in Sorbitol (72 hours, 37°C.)	100	0
Acid and Gas in Rhamnose (48 hours, 37°C.)	100	0
Voges-Proskauer reaction (72 hours, 37°C.)	0	100
Gelatin liquefaction (96 hours, 37°C.)	0	88.8
Milk coagulated (72 hours, 37°C.)	0	84.5
Agglutinated by *Aerob. macerans* serum (2 sera tested)	100	0
Agglutinated by *Aerob. polymyxa* 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.

in the standard fermentation tube. The synonomy of the species and the characteristics for differentiation are listed below.

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

Voges-Proskauer reaction positive; neither acid nor gas produced from rhamnose and sorbitol; no growth at 42° to 45°C., but good growth at 20°C. and slow growth at 13°C.

2. *Aerobacillus macerans* (Schardinger) Donker 1926
   Syn: *Bacillus macerans* Schardinger 1905
   Bacillus acetoethylicus Northrop 1919
   Aerobacillus acetoethylicus (Northrop) Donker 1926

   Voges-Proskauer reaction negative; acid and gas produced from rhamnose and sorbitol; good growth at 42° to 45°C. but little or no growth at 20°C.

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