STUDIES ON AEROBIC SPORE-BEARING NON-PATHOGENIC BACTERIA

PART II

From the Laboratory of Hygiene and Bacteriology, Johns Hopkins University

SPORE-BEARING BACTERIA IN DUST

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Spore-bearing organisms from dust were obtained by rubbing moist sterile swabs over various dust-laden surfaces, transferring the material thus obtained to melted agar and then heating to 90°C. for fifteen minutes to destroy all non-sporulating bacteria. Plates were then poured in the usual way and different colonies selected for study and identification. In many instances the cultures had to be replated a number of times before the purity of the strain was established, so closely do the spores adhere to each other. In general the most prolific source of the spore-bearing organisms was dust which had lain undisturbed for long periods of time as in closets or on high shelves. Dust particles circulating in the air seemed relatively free from spore-bearing bacteria but an increase of these species was always noted with an increased velocity of the wind. Dust from moist surfaces allowed to dry down and from surfaces exposed to direct sunlight contained few spore-bearers. Numerous strains were obtained from the dust found on books. Some 312 cultures were studied and the types (as established in accord with results of the previous work on milk and on miscellaneous cultures) were found to be distributed as follows.

Bacillus cereus Frankland	93
Bacillus subtilis (Ehrenberg) Cohn	71
Bacillus vulgatus (Flügge) Trevisan	46
(*Bacillus mesentericus vulgatus* Flügge.)	
Bacillus megatherium De Bary	39

Bacillus petasites Gottheil	21
Bacillus mesentericus (Flügge) Migula	17
(*Bacillus mesentericus fuscus* Flügge.)	
Bacillus aterrimus Lehmann & Neumann	8
(*Bacillus mesentericus niger* Lunt.)	
Bacillus fusiformis Gottheil	4
Bacillus brevis Migula	2
Bacillus albolactus Migula	1
Bacillus terminalis Migula	1

In addition to these previously established types, on four occasions an organism was encountered giving the same reactions as the species discovered by Prausnitz in Flügge's laboratory and described by Flügge (1886) as *Bacillus ramosus liquefaciens*. This organism was correctly named *Bacillus prausnitzii* by Trevisan (1889). It is distinct from *Bacillus mycoides* of Flügge but the use of the term, "Würzelbacillus" and the name "Ramosus" for this latter organism by both Eisenberg (1891) and the Franklands (1894) with a coincident description of *Bacillus mycoides* as a distinct species by the latter has led to hopeless confusion. Our investigations show that the majority of organisms of this group, producing felted growths in the depths of agar, correspond in all particulars to *Bacillus mycoides* of Flügge which is probably identical with the "Würzelbacillus" and also with the *Bacillus ramosus* of both Eisenberg and the Franklands. The *Bacillus ramosus liquefaciens* of Flügge is a distinct species which we shall describe under its correct name *Bacillus prausnitzii* Trevisan (syn. *Bacillus ramosus liquefaciens* Flügge).

Two cultures were isolated which exhibited the morphology and general cultural characters of the members of the "mesentericus" group but produced an abundant yellow pigment. At first we were inclined to regard this organism as identical with the species described by Sternberg (1892) as *Bacillus subtilis similis*, but the morphology was so clearly that of the mesentericus type that it was deemed best to describe it as a new variety of *Bacillus mesentericus* to which the varietal name *flavus* is given. On one occasion a culture was obtained which seems to represent *Bacillus ruminatus* of Gottheil. It shows the peculiar porcelain-white growth on agar said by Gottheil to be the prin-

cipal feature distinguishing *B. ruminatus* from *Bacillus megatherium*, which it most closely resembles. The species described originally by Vogel (1897) as *Bacillus mesentericus panis viscosi I*, now known as *Bacillus panis* Migula was found once; and one isolation proved to be a new species belonging to the mycoides group to which we have given the name BACILLUS ADHAERENS. The above list of organisms found in dust must therefore be supplemented by the following types whose descriptions are given below in full.

Bacillus prausnitzii Trevisan	4
(*Bacillus ramosus liquefaciens* Flügge.)	
Bacillus mesentericus variety *flavus* nov. var.	2
Bacillus ruminatus Gottheil	1
Bacillus panis Migula	1
(*Bacillus mesentericus panis viscosi* I Vogel.)	
BACILLUS ADHAERENS, nov. sp.	1

Bacillus prausnitzii Trevisan

This organism was originally described as *Bacillus ramosus liquefaciens* by Flügge. It is sometimes regarded as identical with *Bacillus mycoides* but a culture obtained from the Kral collection in Vienna shows different reactions from those of *Bacillus mycoides*. Several cultures corresponding closely to the Kral culture were isolated from dust. The following description applies to the Kral culture and to our own isolations, as well.

Morphology. In young cultures 6 hours old on plain agar the organisms are homogeneous, have round ends when free and flattened ends when in juxtaposition. They generally occur in chains of 2 to 4 elements and resemble *Bacillus mycoides* in morphology. The single cells measure 0.625 to 0.75 by 3 to 5 microns. On glucose agar they are thicker measuring 0.75 to 1 by 3 to 5 microns. In older cultures, 24 hours, on both plain and glucose agar the protoplasm is converted into globular bodies which take the stain badly. These are especially abundant on glucose agar which also shows long and thick vegetative rods measuring 0.75 to 1 by 4 to 6 microns and peculiar washed-

out organisms which seem to be made up of a fine network or skein of filaments. (Figures 53 and 54.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early appearing in 24 hours on plain and on glucose agar. They are usually central, one to a cell, and are slightly wider than the vegetative rods. The organisms with spores retain their chain formation and later the free spores may also remain attached in chains. The free spores are cylindrical and measure 0.75 to 1 by 1.5 to 2 microns. As they lose their protoplasm they become oval and measure 0.625 to 0.75 by 1 to 1.25 microns.

Agar slant. Profuse spreading dull growth consisting of fine interlacing filaments developing from the central line of inoculation as a rhizoid mass. The early growth is extremely tenacious and extends deeply into the underlying agar. Later the growth becomes finely granular and friable and can be scraped from the medium. In general the appearance on agar is like that of a culture of *Bacillus mycoides.*

Agar stab. Abundant growth along line of inoculation and spreading surface growth.

Agar colonies. Colonies consist of profusely interlacing filaments spreading from opaque centers. They are dull grayish and penetrate the agar, under the surface of which they grow in the medium.

Glucose litmus agar slant. Scanty growth on the surface with a pronounced acid reaction which remains permanent.

Glucose litmus agar colonies. Colonies much the same as those on plain agar but somewhat more profuse, with the filamentous character more pronounced.

Gelatin stab. Progressive funnel-like liquefaction often complete within three days.

Gelatin colonies. Colonies consist of profusely interlacing filaments spreading from opaque centers. They are dull greyish and penetrate the gelatin, under the surface of which they grow as in agar. Each colony is soon surrounded by a zone of liquid gelatin.

Broth. Granular scum and flocculent growth which soon settles to the bottom.

Peptone. Similar granular scum and flocculent sediment.

Potato. Viscous yellowish-gray growth spreading profusely and rapidly over the whole medium.

Litmus milk. Reaction highly acid within 24 hours and a firm coagulation within 48 hours. Peptonization soon begins and proceeds very slowly eventually converting the coagulum to an amber-colored fluid. Coagulation is more rapid in freshly isolated strains.

Blood serum. Profuse moist dull interlacing or mycelioid growth. No peptonization.

Fermentation tubes. Glucose: a flocculent growth in the bowl and a granular scum. Turbidity in closed arm. Reaction acid.

Saccharose: a flocculent growth in the bowl with an acid reaction. Arm usually clear.

Lactose: a similar flocculent growth with an acid reaction. Arm usually clear.

Thermal death point. Spores survive 12 pounds in the autoclave but are killed by 15 pounds. In the Arnold they survive 45 minutes but are destroyed by an hour's exposure.

Bacillus mesentericus variety *flavus* nov. var.

This is a new variety of *Bacillus mesentericus* to which the name *flavus* is given because of the abundant yellow pigment it produces. We have encountered it repeatedly in dust and in soil.

Morphology. Thin homogeneous rods with round ends, in young cultures on plain agar measuring 0.375 to 0.5 by 1.5 to 4 microns. On glucose agar the organisms are a little thicker and longer, measuring 0.5 to 0.75 by 3 to 5 microns. They often grow in long threads measuring 9 to 12 microns in length. Shadow forms are formed early both on plain and on glucose agar. In old cultures especially on glucose agar the long forms tend to curve. (Figures 55, 56 and 57.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form within 24 hours. By the end of the 2nd day the spores are abundant. They arise in the center or towards one end of the organism and bulge the rod but little. The free spores retain rims of protoplasm often greater in quantity at one end than at the other. They resemble the mesentericus spores and measure usually 0.625 by 0.75 to 1.5 microns. As they mature they lose their rims of protoplasm, become more oval and measure 0.5 to 0.625 by 0.75 to 0.875 microns.

Agar slant. Moist, smooth, non-spreading lemon-yellow growth. The lemon-yellow color becomes more pronounced with age.

Agar stab. Profuse granular growth along line of stab with slight irregular outgrowths.

Agar colonies. The colonies are smooth, moist, round, with no tendency to spread, glistening and raised, lemon-yellow in color. There is a definite opaque yellow center while the periphery is translucent and shell-like. Deep colonies have a tendency to spread and become faintly iridescent.

Glucose litmus agar slant. A very scant, moist, granular and slightly yellow growth is produced with a slight acid reaction.

Glucose litmus agar colonies. Colonies similar to those on agar but less profuse. Reaction of medium acid.

Gelatin stab. A very slow liquefaction is produced along the line of stab and a slight yellow tinge is imparted to the medium.

Gelatin colonies. The colonies rest in slight depressions caused by slow liquefaction. They are dark yellow in color, moist, round and smooth. Under the low power of the microscope each colony is seen to be composed of concentric circles of varying densities with lobate edges.

Broth. A slight turbidity appears after a considerable period. The medium clears by sedimentation and the sediment has a slight yellow tinge.

Peptone. A slight turbidity, somewhat less than in broth occurs. This soon settles to the bottom.

Potato. No visible growth.

Litmus milk. No change in reaction even after a long period of time.

Blood serum. A scant, moist, smooth, glistening and yellowish growth is produced. No solution of the medium occurs.

Fermentation tubes. Glucose: a slight turbid growth occurs in the bowl with the production of a slight acidity.

Saccharose: the same appearance. No acid.

Lactose: slight turbidity. No acid.

Thermal death point. Spores survive 18 pounds pressure in the autoclave but are destroyed by 20 pounds. They survive one hours steaming in the Arnold.

Bacillus ruminatus Gottheil 1901

The type "ruminatus" was first described by Gottheil (1901). In morphology and cultural characters it closely resembles *Bacillus megatherium* from which it is distinguished by its porcelain-white growth, particularly in young cultures. The present description applies to an organism found in dust and subsequently in water which corresponds to Gottheil's original description but not however to that given by Chester. We believe that it may properly be called *Bacillus ruminatus.*

Morphology. Homogeneous rods with rounded ends measuring 0.625 to 0.75 by 2.5 to 4 microns in young cultures on plain agar. On glucose agar they are distinctly thicker and longer measuring 0.75 to 1.125 by 2.25 to 5 microns. Rarely, long forms are found on this medium measuring 8 to 10 microns in length. Shadow or washed out forms are common on both plain and glucose agar measuring 1.025 to 1.5 by 3 to 5 microns. Organisms often appear in short chains. (Figures 58 and 59.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early, often in 48 hours, especially when the strains are first isolated. They are central or slightly ex-centric and on sporulation swell or bulge the organisms from which they spring. They measure usually 0.625 by 1.25 to 1.5 microns. Some spores are however somewhat smaller and more oval.

Agar slant. Moderate growth along line of inoculation forming a definite ridge. There is a tendency for the growth to spread to either side and the opacity of the growth decreases towards the periphery which is translucent. The growth is glistening, raised, moist and has a pure white color which becomes more apparent with age.

Agar stab. Growth along the line of inoculation distinctly granular.

Agar colonies. The colonies are very characteristic. Some are round and regular while others show a considerable tendency to spread. They are moist, opaque, raised, glistening and white. The majority show dense centers surrounded by thin areas which are in turn surrounded by translucent shell-like peripheries.

Glucose litmus agar slant. The growth is similar to that on agar although somewhat variable as to amount. The distinct white coloration is also evident. A definite and permanent acidity is produced.

Glucose litmus agar colonies. The colonies correspond closely to those on agar but are less profuse.

Gelatin stab. A fairly rapid progressive cone-like liquefaction occurs.

Gelatin colonies. The colonies rest in a cup-like excavation caused by liquefaction. They are definitely circumscribed and have an opaque center which is surrounded by a less dense grayish area. This in turn is enclosed by a more dense grayish ring. Outside this ring the opacity decreases towards the periphery.

Broth. A fine fragile pellicle is formed with some turbidity. The medium clears by sedimentation.

Peptone. Similar scum but less marked turbidity.

Potato. Cream-white moist profuse growth developing in 24 to 48 hours.

Litmus milk. Within 24 hours a slight acid reaction occurs and the milk often shows a distinct thickening at the bottom. The coagulation is not definite however and peptonization begins usually within 48 hours. As it advances a clear zone of amber-colored fluid is found at the upper part of the milk tube. This gradually increases until all the milk is peptonized.

Blood serum. Fairly profuse moist glistening smooth whitish growth. Some softening of the serum but no definite liquefaction.

Fermentation tubes. Glucose: a slight turbidity in the bowl and neck with a reduction of the litmus in the closed arm. Reaction acid.

Saccharose: a similar growth with an acid reaction.

Lactose: only a slight turbidity develops with an alkaline reaction.

Thermal death point. The spores survive 20 pounds pressure in the autoclave but are destroyed by 22 pounds. They survive one hours steaming in the Arnold.

Bacillus panis Migula 1900

This organism was originally described by Vogel (1897) as *Bacillus mesentericus panis viscosi I.* It has been found in Baltimore, but once, in dust.

Morphology. When first isolated this organism showed only encapsulated forms, the capsules staining readily with gentian violet. As the organism was cultivated on artificial media in the laboratory it lost its capacity of forming easily-stained capsules but continued to manufacture a quantity of viscous material which gave a characteristic appearance to the cultures. In young cultures on plain agar, 6 to 24 hours old, the organisms are small and homogeneous with round to flattened ends, measuring 0.375 to 0.5 by 1.5 to 3 microns. They show no appreciable difference in thickness on glucose agar, but tend to show long forms measuring 5 to 6 microns in length. Occasional shadow forms are seen measuring 0.75 to 1 by 1.5 to 4 microns. (Figures 60, 61, and 62.)

Motility. No motility has thus far been demonstrated.

Staining properties. Gram-positive.

Spore formation. When first isolated, spores were formed early, often in 24 to 48 hours. After long cultivation in the laboratory, they appear in the cultures only after 6 to 8 days growth. They are formed in the centers or towards one end of the rods and are

typical mesentericus spores. They retain definite rims of protoplasm at times concentrated at one end of the spore and measure 0.375 to 0.5 by 1 to 1.25 microns. As they lose their rims of protoplasm they become more oval and measure about 0.75 by 1 micron.

Agar slant. Growth scanty in 24 hours, then becomes slightly raised, finely wrinkled, translucent, non-spreading and viscous. When older the growth has a tendency to become somewhat dry and gray and is easily scraped off. On highly acid agar the growth is more profuse within 24 hours but is not viscous.

Agar stab. Slight granular growth along the line of inoculation with occasionally a slight budding out from the stab.

Agar colonies. Colonies small, drop-like, slightly irregular, showing little or no tendency to spread, glistening, elevated and viscous. In some cases a scum-like covering which enclosed clear gelatinous material was produced about each colony.

Glucose litmus agar slant. Moderate non-spreading growth within 24 hours. The surface of the growth has a sort of honeycombed appearance caused by fine interlacing wrinkles. It is also viscous and is somewhat bluish-gray in color. The reaction is definitely acid within 24 hours. This acidity is followed by a reduction of the litmus and a gradual return to alkalinity.

Glucose litmus agar colonies. The colonies correspond closely to those on plain agar but usually attain somewhat greater dimensions.

Gelatin stab. Rapid funnel-like and progressive liquefaction. Complete liquefaction results with the formation of a tenacious grayish scum.

Gelatin colonies. The colonies on gelatin plates rest in cup-like excavations caused by rapid liquefaction. A definite brown center with a surrounding grayish granular area is evident in each colony under the low power of the microscope.

Broth. A slight turbidity is produced within 24 hours with the beginning formation of a scum. The scum later is finely granular and is formed of discrete colonies. The medium clears itself by sedimentation.

Peptone. Reaction the same as that in broth.

Potato. Growth finely wrinkled, grayish and viscous. The wrinkles appear wave-like. When older the growth loses its viscosity and becomes dry and granular.

Litmus milk. A gradual clearing from the top due to progressive peptonization occurs within 24 hours. Within 48 hours peptonization is generally completed. The remaining fluid has a port-wine color but becomes amber-colored after a variable period.

Blood serum. Smooth, moist, glistening and viscous growth. Within 24 hours some solution of the medium occurs along the line of inoculation (a trough-like excavation). The medium may be entirely dissolved within 2 weeks, and a tenacious scum may be formed.

Fermentation tubes. Glucose: finely granular scum is formed which generally breaks up into large flakes. A flocculent growth is present in the bowl. The reaction is definitely acid.

Saccharose: the growth is the same and the reaction also acid.

Lactose: the growth is also abundant with more pronounced scum-formation. Reaction alkaline.

Thermal death point. The spores survive 10 pounds in the autoclave but are destroyed by 15 pounds pressure. They survive 30 minutes steaming in the Arnold but are destroyed by one hours exposure.

BACILLUS ADHAERENS nov. sp.

This organism has been encountered but once, in dust. It is apparently a new species.

Morphology. Slender long rods with homogeneous protoplasm and flat ends, growing usually in long curved chains made up of 18 to 20 elements. In young cultures on plain agar the individual cells measure 0.375 to 0.5 by 1.5 to 4 microns. Some longer forms, 6 microns in length, may also be found. On glucose agar the organisms are homogeneous, measure 0.625 to 0.75 by 3 to 5 microns, but are often longer measuring 6 to 8 microns. In older cultures (4 days) many globular bodies occur on glucose agar. They resemble the globular bodies seen in *Bacillus cereus.* (Figures 63 and 64.)

Motility. No motility has ever been observed, even in very young cultures.

Staining properties. Gram-positive.

Spore formation. When first isolated this species formed spores in 24 hours. After long artificial cultivation spores are formed only after 4 to 5 days growth on both plain and glucose agar. They are usually subterminal but may be central. The rods swell appreciably before sporulation, sometimes in the center and sometimes at the ends. The free spores are oval and measure 0.625 to 0.75 by 0.875 to 1 micron. They often remain fastened to each other in long chains. Frequently a bit of protoplasm remains attached to the spore which then resembles a tennis racket with its handle.

Agar slant. In early growth (18 hours) this species slightly resembles *Bacillus mycoides*. The line of inoculation shows a distinct ridge from which shoot out fine interlacing filaments. These adhere closely to and grow into the agar. Considerable puckering of these interlacing filaments causes a roughened leathery appearance on the surface. The early growth is moist and slightly glistening but these properties are soon lost. The edges of the growth are serrated, with little or no tendency to spread. A brownish color is found in old cultures throughout the entire medium.

Agar slant. Profuse growth along the line of inoculation and out into the medium. This has the appearance of an inverted fir tree.

Agar colonies. The colony is very characteristic. It first appears like a small colony of *Bacillus mycoides*, but within 24 hours the filaments seem to swell and produce a somewhat corrugated surface with a very definite, elevated and yellow-brown center. The entire colony adheres closely to the agar and gradually grows into it.

Glucose litmus agar slant. The growth is similar to that on plain agar but is very scanty. A definite and permanent acidity is produced.

Glucose litmus agar colonies. Colonies in this medium are considerably smaller than those on plain agar and have the same general appearance.

Gelatin stab. In gelatin, growth is slow and a very slow funnel-like liquefaction is produced.

Gelatin colonies. They appear coarsely granular, slightly raised with definite yellow-brown centers. The centers are coarsely flocculent under the low power of the microscope. The colonies are surrounded by a slight area of liquefaction.

Broth. A slight turbidity is produced and a definite scum is formed which settles to the bottom.

Peptone. Growth similar to that in broth.

Potato. A fairly profuse grayish-white moist growth. When the medium is dry the growth is scale-like.

Litmus milk. No change is noticed within 24 hours. After 48 hours a slowly progressive peptonization occurs. The medium becomes amber-colored.

Blood serum. A fine-grained leather-like growth occurs, dull gray and adherent to the medium. Later this is easily scraped off. No solution of the serum occurs.

Fermentation tubes. Glucose: a flocculent growth occurs in the bowl and extends into the closed arm. Reaction acid.

Saccharose: turbidity in bowl. Arm clear. Reaction neutral or slightly acid.

Lactose: turbidity in bowl. Arm clear. Reaction not changed.

Thermal death point. The spores resist 18 pounds pressure in the autoclave but are destroyed by 20 pounds. They survive one hour's steaming in the Arnold.

SPORE-BEARING ORGANISMS IN WATER

BY C. A. LAUBACH

The spore-bearing organisms in water were obtained by passing the tap water in the laboratory through Berkefeld filters under pressure for a period of three days, washing the filters in sterile salt solution, heating the washings to 80°C. for 15 minutes and then plating. Ten samples were obtained by this method and 313 organisms studied. The species previously established from studies of milk and dust were found as follows: