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JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

PROGRESS REPORT

Microorganism Study. JPL Contract No. 950783

Prof. W. B. Bollen, Microbiologist

Oregon State University, Corvallis, Oregon

April 9, 1965

This work was performed for the Jet Propulsion Laboratory, California Institute of Technology, sponsored by the National Aeronautics and Space Administration under Contract NAS7-100.

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Work on 19 Bacillus cultures has been completed, except for precise growth temperature range determinations which await modification and adaptation of a borrowed temperature gradient apparatus. Studies on the other cultures submitted in the first two lots are nearly complete and work has been started on the third collection delivered February 25. A graduate assistant has been assigned to the project and is now giving full time to the systematic studies. Progress will now be more rapid.

A descriptive chart for each Bacillus is presented herewith. The species have been identified according to Bergey's Manual, and Smith, Gordon, and Clark's (Aerobic Sporeforming Bacteria) characters considered most significant. Important variations are indicated.

Each descriptive chart is supplemented by an attached sheet giving a more complete characterization of the colony characteristics.

The morphology descriptions are supplemented with photomicrographs of nigrosin, Gram, and flagella stains, taken at 1000X. Each division on the micrometer scale has a value of 0.5 micron. Dimensions given are from measurements of 10 or more cells on the nigrosin slide. Size range as well as the mean is shown under Cell Morphology. Where colors are named and preceded by a number and letters--e.g., 2ba Pearl-- this indicates the identification given in the Color Harmony Manual, published by the Container Corporation of America. I have found this system to be accurate, rapid, and adaptable to microbial cultures and it is the most comprehensive set of color standards available.

Other details on the descriptive charts are self-explanatory. Ages, temperatures and kind of media are indicated. Unless otherwise stated, trypticase soy media were used.

A full discussion of these isolants, their characteristics, and the methods will be presented at a later date. Several of the cultures have been very frustrating to work with because of apparent contamination. Now, however, we believe the differently appearing colonies are evidence of dissociation. Absolute proof of this would require single cell isolations, but we do not have the necessary micromanipulators.

W. B. Ballou

4-9-65

Name of organism Bacillus megaterium Studied by Dr. W.B. Bollen Culture No 10-2-B

Source Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*)

Sketches X1000

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28 °C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: – Flagella: Peritrichous

Size: 1.9 x 3.6μ 1.5-3.5 x 1.5-6.5μ Irregular forms:

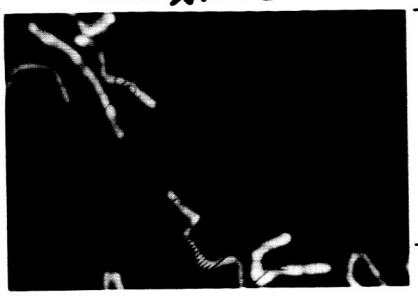
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to eccentric, terminal, subterminal.*

Nigrosin



1 div. = 0.5μ

STAINING CHARACTERISTICS

Gram: + Age: 24hr. Method: Kopeloff's (modified)

Special stains:

AGAR STROKE Age: 5 day Temp. 28°C.

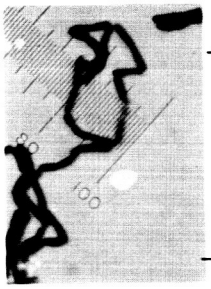
Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: ; *fluorescent, iridescent, photogenic.*

Gram stain



24 hr 48 hr

AGAR COLONIES Age: 3 day Temp. 28 °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 24 hour Temp. 28°C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 24 hour Temp. 25°C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 2db Ivory

Soybean agar slant: Growth abundant

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	Mannitol
Acid in 3 days	+				
Acid in days					
Gas in 3 days	–				
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus

	Days					
	4		30			
Reaction	=		=			
Acid curd	=		=			
Rennet curd	=		=			
Peptonization	+		+			
Reduction (before coagulation)	=		=			

ACTION ON NITRATES

Medium: 1%KNO3 broth Temp. 28 °C.

Nitrite: + 2 d. ; ..... d. ; ..... d.

Gas (N): - 3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 days

Method: Kovac's Temp. 28 °C.

Indole: *present*, *absent*.

HYDROGEN SULPIDE PRODUCTION

Medium: Lead Acetate & Thio-iron Age 10 days

H_2S: *present*, *absent*. Temp. 28 °C.

TEMPERATURE RELATIONS

Growth in refrigerator (°C.): *present*, *absent*.

Growth at room temperature (°C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*.

RELATION TO FREE OXYGEN

Medium: Dextrose Trypt-soy Age: 10 days

Method: Shake Tubes Temp. 28 °C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative (+ in Bergey)
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive
Metabolism: Oxidative - Glucose
Acetyl methyl carbinol: Negative

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Negative

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 10B
Trypticase-soy agar
3 Day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 4mm

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 2ba (Pearl)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilus Studied by Dr. M. P. Boller Culture No. 14-2-B

Source Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*)

Sketches

CELL MORPHOLOGY Medium: Tryptic case soy agar Temp. 28 °C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

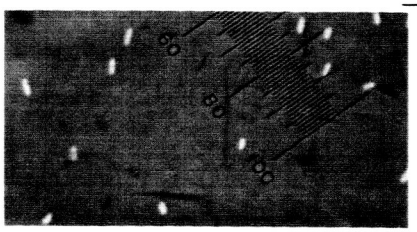
Motility in broth: + Flagella: Peritrichous

Size: 0.65 x 2.1μ Irregular forms:
0.5 - 0.75 x 1.5 - 3.0μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:
Shape: *spherical, ellipsoid, cylindrical.*
Position: *central to excentric, terminal, subterminal.*

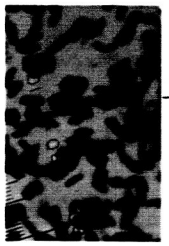
Nigrosin



STAINING CHARACTERISTICS

Gram: + Age: 24hr. Method: Kopeloff's (modified)

Special stains:



24 hr

48hr

AGAR STROKE Age: 5 day. Temp. 28 °C.

Amount of growth: *scanty, moderate, abundant.*
Form: *filiform, echinulate, beaded, spreading, rhizoid.*
Consistency: *butyrous, viscid, membranous, brittle.*
Chromogenesis: Brick red *fluorescent, iridescent, photogenic.*

Gram stain

AGAR COLONIES Age: 3 day Temp. 28 °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*
Elevation: *effuse, flat, raised, convex.*
Surface: *smooth, contoured, radiate, concentric, rugose.*
Margin: *entire, undulate, erose, filamentous, curled.*
Density: *opaque, translucent.*

NUTRIENT BROTH Age: 1 day Temp. 28 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*
Subsurface growth: *none, turbid, granular.*
Amount of growth: *scanty, moderate, abundant.*
Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 1 day Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*
Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 6pe Dk. Lacquer Red
Soybean agar slant: No growth

FERMENTATION Temp. 25 °C.

Medium Nutrient broth Carbohydrate: Indicator BCP 1%	Glucose	Lactose	Sucrose		
Acid in 3 days	=	=	=		
Acid in days					
Gas in 3 days	=	=	=		
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 14					
Reaction	=					
Acid curd	=					
Rennet curd	=					
Peptonization	=					
Reduction (before coagulation)	=					

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp 28 °C.
Nitrite: -10 d. ; ..... d. ; ..... d.
Gas (N): -10 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 da.
Method: Kovac's Temp 28 °C.
Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate & Age: 10 da.
H_2S: *present*, *absent*. Thio iron Temp 28 °C.

TEMPERATURE RELATIONS

Growth in refrigerator (°C.): *present*, *absent*.
Growth at room temperature (28 °C.): *present*, *absent*.
Growth at 37° C.: *present*, *absent*.
Growth at 50° C.: *present*, *absent*.

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Trypt. Soy Age: 10 day
Method: Kovac's Temp 28 °C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Oxidative

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 14b

Trypticase soy agar 3 day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 2 mm

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 5pe Terra Cotta

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilus Studied by Dr. W.B. Bollen Culture No. 14-2-D

Source Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium Trypticase soy agar Temp. 28°C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous —

Size: 0.5 × 1.8μ Irregular forms:
0.5 × 1.0 to 2.5μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

N: gross

STAINING CHARACTERISTICS

Gram: + Age: 24hr Method: Kopeloff's (modified)

Special stains:

24hr 48hr

AGAR STROKE Age: 5 day Temp. 28°C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: Red brown; *fluorescent, iridescent, phytogenic.*

Gram stain

AGAR COLONIES Age: 3 day Temp. 28°C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 24 hr. Temp. 28°C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 24 hr. Temp. 25°C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 6pe Dark Lacquer Red

Soybean agar slant: No growth

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP	Glucose	Lactose	Sucrose		
Acid in 3 days	=	=	=		
Acid in days					
Gas in 3 days	=	=	=		
Gas in days					

ACTION ON MILK Temp. 28°C.

Indicator: Litmus	Days					
	14					
Reaction	=					
Acid curd	=					
Rennet curd	=					
Peptonization	=					
Reduction (before coagulation)	=					

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp. 28 °C.

Nitrite: .....d. ; .....d. ; -10 d.

Gas (N): .....d. ; .....d. ; -10 d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 da.

Method: Kovac's Temp. 28C.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate & thio iron Age: 10 da. Temp. 28 °C.

H_2S: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (°C.): *present*, *absent*.

Growth at room temperature (28°C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*.

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Trypt. Soy Age: 10 da.

Method: Shake tubes Temp 28 °C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Oxidative

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 14d
Erypticase soy agar
3 day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 2 mm

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 5pe Serra Cotta

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilus Studied by Dr. W.B. Bollen Culture No. 15-2-C

Source Habitat Soil Date

Descriptions (*Underscore required terms.*)	Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28°C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.65 × 2.33M Irregular forms:
0.5-0.75 × 1.5-3.5M

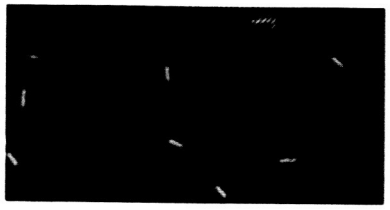
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

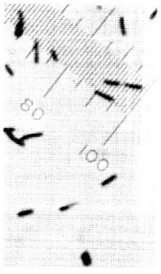
Nigrosin



STAINING CHARACTERISTICS

Gram: + Age: 24hr. Method: Kopeloff's (modified)

Special stains:



24 hr

48 hr

AGAR STROKE Age: 5 day Temp 25 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: Red brown *fluorescent, iridescent, photogenic.*

Gram stain

AGAR COLONIES Age: 3 day Temp. 28°C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 24 hour Temp. 28°C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 24 hr. Temp. 25°C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 6pe Dark Lacquer Red

Soybean agar slant: No growth

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth
Carbohydrate: 1 %
Indicator: BCP

	Glucose	Lactose	Sucrose		
Acid in 3 days	–	–	–		
Acid in days					
Gas in 3 days	–	–	–		
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 14					
Reaction	=					
Acid curd	=					
Rennet curd	=					
Peptonization	=					
Reduction (before coagulation)	=					

ACTION ON NITRATES

Medium: 1%KNO3 broth Temp 28 °C.

Nitrite: .....d. ; .....d. ; -10d.

Gas (N): .....d. ; .....d. ; -10d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day

Method: Kovac's Temp 28 °C.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio iron Age: 10 da. Temp 28 °C.

H_2S: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present*, *absent*. (+ -)

Growth at room temperature (28 °C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 60° C.: *present*, *absent*. (+ -)

RELATION TO FREE OXYGEN- Catalase: Positive

Medium: Dextrose Trypt.soy Age: 10 day

Method: Shake tubes Temp. 28 °C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

ADDITIONAL TESTS

Pasteurization survival, 80°C. 10 minutes: Positive

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Oxidative

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Negative
Sucrose - Negative
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 15c

Trypticase soy agar 3 day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 2 mm

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 5pe Teresa Cotta

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus subtilis var. niger Studied by Dr. W.B. Bollen Culture No. 160

Source White Mountain Habitat Soil Date

Descriptions (*Underscore required terms.*)	Sketches

CELL MORPHOLOGY Medium: Trypticase soy Temp. 28 °C.

Vegetative cells: Age: 24hrs

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.75 x 2.8μ Irregular forms:

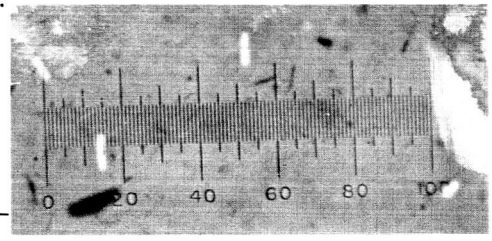
0.75 x 2.0-4.0μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

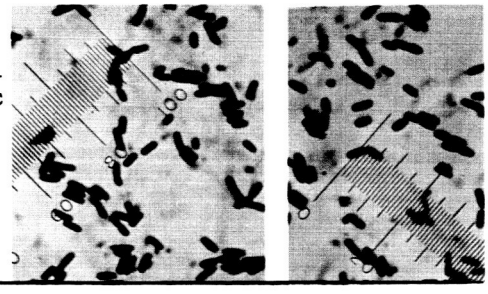


NIGROSIN

STAINING CHARACTERISTICS

Gram: + − Age: 24hr Method: Modified Kopeloff

Special stains:



48hrs.

AGAR STROKE Age: 24 hrs. Temp. 25 °C

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: ; *fluorescent, iridescent, photogenic.*

Brown-black water soluble pigment 72 hours.

24 hrs.

GRAM STAIN

AGAR COLONIES Age: Temp. °C.

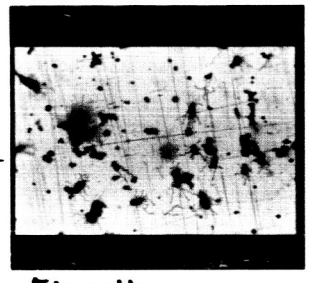
Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*



Flagella

NUTRIENT BROTH Age: 2 days (islands) Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 24 hours Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant:	2ge	Covert Tan
Soybean agar slant:	31g	Adobe Brown - abundant
Glucose nitrate slant:		- scant growth
Tyrosine agar slant:		Black pigment
Fat agar:	3nl	Dark Brown

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	−	−	+	−	
Acid in 8 days	−	−	+	−	
Gas in 2 days	−	−	−	−	
Gas in 8 days	−	−	−	−	

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 2	4				
Reaction	=	ALKALINE				
Acid curd						
Rennet curd						
Peptonization	+					
Reduction (before coagulation)	+					

ACTION ON NITRATES

Medium: 1%KNO3 broth Temp. 28°C.

Nitrite: + 3 d. ; ..... d. ; ..... d.

Gas (N): - 3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 days

Method: Kovac's Temp. 28°C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio iron Age: 10 days

H_2S: *present, absent.* Temp. 28°C.

TEMPERATURE RELATIONS

Growth in refrigerator (10C.): *present, absent.*

Growth at room temperature (28C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.* (sl.)

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a Age: 10 days

Method: Shake Tubes Temp. 28°C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermenter (glucose and sucrose) anaerogenic
Nonoxidizer-nonfermenter (lactose and xylose)

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 16c

Septicase - soy agar
5 day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 2mm

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, (rough) wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3pa (Chocolate Brown)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

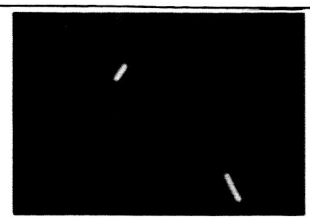
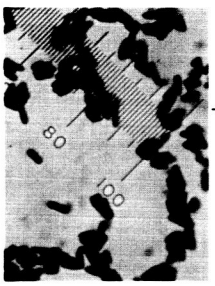
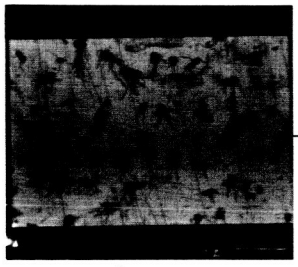
a. Size, mm

b. Shape

c. Color

Name of organism Bacillus subtilis var. niger Studied by Dr. W.B. Bollen Culture No. 1601

Source White Mountain Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy Age: 24hrs Temp. 28 °C. Vegetative cells: Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: + Flagella: Peritrichous Size: 0.96 x 2.8 μ Irregular forms: 0.75 - 1.0 x 2.0 - 4.5 M Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.* nor swollen Nigrosin	
STAINING CHARACTERISTICS Gram: + − − Age: 24hrs Method: Kopeloff's (modified) Special stains:	 24 hr  48hr
AGAR STROKE Age: 24 hrs. Temp. 25°C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: White ; *fluorescent, iridescent, photogenic.* Gram Stain	
AGAR COLONIES Age: Temp. °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	 Flagella
NUTRIENT BROTH Age: 2 days (islands) Temp. 25°C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 24 hrs Temp 25 °C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.* (7)	
OTHER MEDIA Age: Temp. °C. Potato slant: 31e Bisque Soybean agar slant: 31g Adobe Brown Glucose Nitrate slant: scant growth Tyrosine agar slant: Black Fat agar: 31g to white (mainly white)	

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth
Carbohydrate: 1 %
Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	=	+	=	
Acid in days					
Gas in 2 days	−	−	−	−	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days					
		2				
Reaction		−				
Acid curd						
Rennet curd						
Peptonization		+				
Reduction (before coagulation)		+				

− (alkaline)

ACTION ON NITRATES

Medium: 1%KNO3 broth Temp. 28c.

Nitrite: - 3 d. ; ..... d. ; ..... d.

Gas (N): - 3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 days

Method: Kovac's Temp. 28c.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio iron Age: 10 days

H_2S: *present*, *absent*. Temp. 28c.

TEMPERATURE RELATIONS

Growth in refrigerator (10 °C.): *present*, *absent*.

Growth at room temperature (28 C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*.

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a Age: 10 days

Method: Shake tubes Temp. 28c.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

Pasteurization survival, 80°C 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermenter - anaerogenic (Glucose and Sucrose)
Nonoxidizer - nonfermenter (Lactose and Xylose)

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 16C1
Trypticase soy agar
5 day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 7 mm

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3gc St. San

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilus Studied by Dr. W.B. Bollen Culture No. 18

Source White Mountain Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 30 °C.

Vegetative cells: Age: 24hrs.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.85 x 2.15 μ Irregular forms:

0.75 - 1.0 x 1.6 - 3.5M

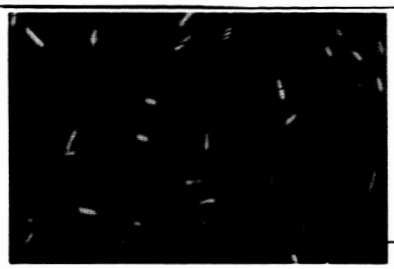
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin



STAINING CHARACTERISTICS

Gram: + − Age: 24 hr Method: Kopeloff's (modified)

Special stains:

AGAR STROKE Age: 24 hour Temp. 25 °C.

Amount of growth: *scanty, moderate, abundant.*

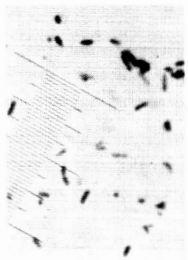
Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: ; *fluorescent, iridescent, photogenic.*

Gram stain

24 hr



48 hr

AGAR COLONIES Age: 2 day Temp. 28 °C.

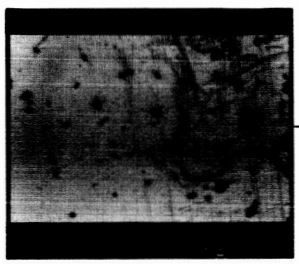
Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*



Flagella

NUTRIENT BROTH Age: 12 days Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.* islands

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

F

GELATIN STAB Age: Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 21e Mustard

Soybean agar slant: 3ca Pearl Pink

Fat agar: Clear to lt. yellow

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth

Carbohydrate: 1 %

Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	=	+	=	
Acid in days					
Gas in 2 days	−	−	−	−	
Gas in days					

ACTION ON MILK Temp. 25 °C.

Indicator: Litmus

	Days					
	3	5	7			
Reaction	↓	=	−			
Acid curd	=	=	=			
Rennet curd	=	+	+			
Peptonization	+	+	+			
Reduction (before coagulation)	+	+	+			

ACTION ON NITRATES

Medium: 1%KNO_3 broth Temp 28 °C.

Nitrite: .....d. ; .....d. ; -3 d.

Gas (N): .....d. ; .....d. ; -3 d.

INDOLE PRODUCTION

Medium: Tryptophan Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate & Thio iron Age: 10 day Temp. 28°C.

H_2S: *present*, *absent*.

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a Age: 10 day

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present*, *absent*.

Growth at room temperature (28 °C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermenter - anaerogenic - Glucose, Sucrose, and Xylose
Nonoxidizer-nonfermenter - Lactose

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 18
Syptic case - soy agar
2 days

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 2mm

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 2Eb (Bamboo)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus cereus Studied by Dr. W.B. Bollen Culture No. 18-2-C

Source White Mountain Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 30 °C.

Vegetative cells: Age: 24hrs

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 2 x 4.7μ Irregular forms:
2 x 3.0-7.0μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin

STAINING CHARACTERISTICS

Gram: + Age: 24 hr Method: Kopeloff's (modified)

Special stains:

24 hr

48 hr

AGAR STROKE Age: 5 day Temp. 28 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: white ; *fluorescent, iridescent, photogenic.*

Gram Stain

AGAR COLONIES Age: 6 day Temp. 28 °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, coloured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 24 hour Temp. 28c.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 24 hour Temp. 25c.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 2ec Biscuit

Soybean agar slant: Abundant growth

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP	Glucose	Lactose	Sucrose		
Acid in 1 days	+				
Acid in days					
Gas in 1 days	−				
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 4	10	14			
Reaction	=	=	−			
Acid curd	=	=	=			
Rennet curd	=	=	=			
Peptonization	=	+	+			
Reduction (before coagulation)	+	+	+			

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp. 28°C.

Nitrite: .....d. ; .....d. ; +2 d.

Gas (N): .....d. ; .....d. ; -2 d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate & Age: 10 da.

H_2S: *present, absent.* Thio iron Temp. 28°C.

TEMPERATURE RELATIONS

Growth in refrigerator (°C.): *present, absent.*

Growth at room temperature (28°C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

RELATION TO FREE OXYGEN - Catalase: Negative

Medium: Dextrose Trypt. soy a Age: 10 da.

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Nonoxidizer-nonfermenter - Glucose

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Negative

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 18c
Trypticase soy agar
6 days.

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 5 m.m

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 1½ca (Cream)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilus Studied by Dr. W.B. Bollen Culture No. 19

Source White Mountain Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 30 °C.

Vegetative cells: Age: 24hr

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.7-1 x 2.5μ 0.9-1.0 x 1.5-3.0μ Irregular forms:

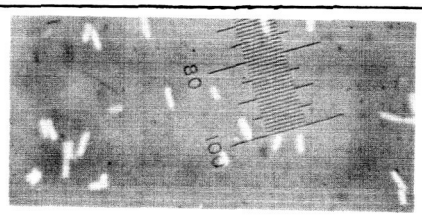
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin

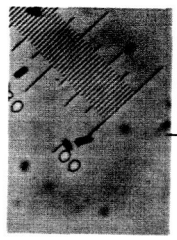


STAINING CHARACTERISTICS

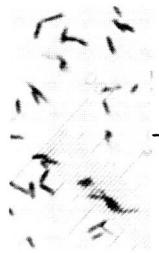
Gram: + − Age: 24hr. Method: Kopeloff's (modified)

Special stains:

Gram Stain



24 hr



48 hr

AGAR STROKE Age: 24 hr. Temp. 25 °C

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: white ; *fluorescent, iridescent, photogenic.*

AGAR COLONIES Age: 5 day Temp. 28 °C.

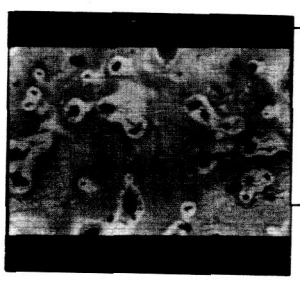
Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*



Flagella

NUTRIENT BROTH Age: 2 day Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 5 day Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 3ie Camel

Soybean agar slant: 3ca Pearl pink

Fat agar: clear with yellow-orange tint scant to moderate

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: % Indicator: BCP 1

	Glucose	Lactose	Sucrose	Xylose	
Acid in 12 days	+	=	+	=	
Acid in days					
Gas in 12 days	−	−	−	−	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 3	14				
Reaction	=	−				
Acid curd	=	=				
Rennet curd	=	=				
Peptonization	+	+				
Reduction (before coagulation)	+	+				

ACTION ON NITRATES
Medium: 1% KNO_3 broth Temp. 28°C.
Nitrite: .....d. ; .....d. ; -3.....d.
Gas (N): .....d. ; .....d. ; -3.....d.

INDOLE PRODUCTION
Medium: Tryptophan broth Age: 10 day
Method: Kovac's Temp. 28°C.
Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION
Medium: Lead acetate & Thio iron Age: 10 day Temp. 28°C.
H_2S: *present*, *absent*.

TEMPERATURE RELATIONS
Growth in refrigerator (10 °C.): *present*, *absent*.
Growth at room temperature (28 °C.): *present*, *absent*.
Growth at 37° C.: *present*, *absent*.
Growth at ~~50~~ 45 C.: *present*, *absent*. (+-)

RELATION TO FREE OXYGEN - Catalase: Positive
Medium: Dextrose Nutrient A Age: 10 day
Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

ADDITIONAL TESTS

Pasteurization survival, 80°C 10 minutes: Positive

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermentative - anaerogenic - Glucose and Sucrose
Nonoxidative-nonfermentative - Lactose and Xylose

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 19
Staphylococcus soy agar 5 days.

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 3mm.

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 4 ic Pastel Orange some colonies white and mixed

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilis Studied by Dr. M.B. Bollen Culture No. 19A1

Source White Mountain Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy Temp. 28 °C.

Vegetative cells: Age: 24hr

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 1.0 x 2.3μ Irregular forms:
1.0 x 2.0-3.0μ

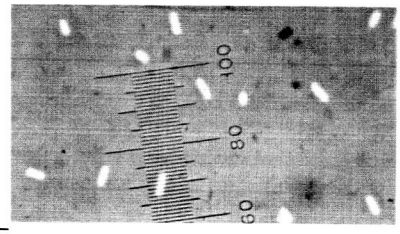
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: 60Hr.

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

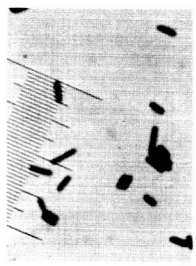
Nigrosin

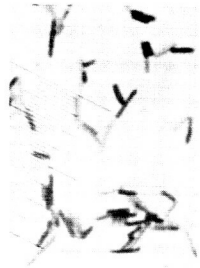


STAINING CHARACTERISTICS

Gram: + Age: 24 Method: Kopeloff's (modified)

Special stains:





24hr 48hr

AGAR STROKE Age: 24 hr. Temp. 25 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: white ; *fluorescent, iridescent, photogenic.*

Gram Stain

AGAR COLONIES Age: 5 da. Temp. °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 2 day Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

(12da)

GELATIN STAB Age: 48 hr Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant:		Black pigment
Soybean agar slant:	3ie	Camel
Glucose nitrate slant:		Clear to white shiny (moderate growth)
Tyrosine agar slant:		Black water soluble pigment
Fat agar:	2ca	Lt. Ivory

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	=	=	+	=	
Acid in days					
Gas in 2 days	=	=	=	=	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus

	Days 3	5	7			
Reaction			−			
Acid curd						
Rennet curd		+				
Peptonization	+					
Reduction (before coagulation)	+					

ACTION ON NITRATES

Medium: 1%KNO_3 broth Temp. 28°C.
Nitrite: -3 d. ; ..... d. ; ..... d.
Gas (N): -3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day
Method: Kovac's Temp. 28°C.
Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio iron Age: 10day
H_2S: *present*, *absent*. Temp. 28°C.

RELATION TO FREE OXYGEN - Catalase: Negative

Medium: Dextrose Nutrient a Age: 10 day
Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present*, *absent*. ( )
Growth at room temperature (28°C.): *present*, *absent*.
Growth at 37° C.: *present*, *absent*.
Growth at 50° C.: *present*, *absent*.

Pasteurization survival , 80°C 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermenter - anaerogenic (Glucose and Sucrose)
Nonoxidizer-nonfermenter (Lactose and Xylose)

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 1941
Trypticase soy agar
5 days

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 4mm

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 2ca St. Ivory

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilus Studied by Dr. W.B. Bollen Culture No. 19A2

Source Habitat Soil Date 4-3-65

Descriptions (*Underscore required terms.*) | Sketches.

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28 °C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.65 x 2.95μ Irregular forms:
0.5 -0.75 x 1.5-4.0μ

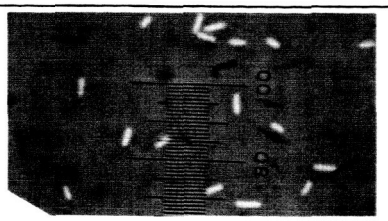
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin



STAINING CHARACTERISTICS

Gram: +− Age: 24hr Method: Kopeloff's (modified)

Special stains:

24 hr

48 hr

AGAR STROKE Age: 24 hr. Temp. 25 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: ; *fluorescent, iridescent, phylogenic.*

Gram Stain

AGAR COLONIES Age: 7 day Temp. 25 °C.

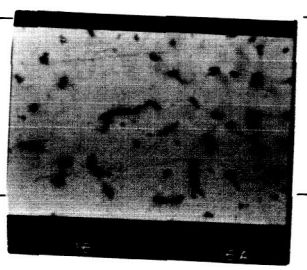
Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*



NUTRIENT BROTH Age: 2 day Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

Flagella

GELATIN STAB Age: 2 day Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 3le Cinnamon

Soybean agar slant: 2db Ivory (abundant)

Fat agar: scant

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth 1% Carbohydrate: Indicator: BCP	Glucose	Lactose	Sucrose		
Acid in 1 days	+	=	+	−	
Acid in 12 days	+	+	+	−	
Gas in 1 days	=	=	=	=	
Gas in 12 days	=	=	=	=	

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 8	35				
Reaction	=	+St.				
Acid curd	=	=				
Rennet curd	=	=				
Peptonization	=	=				
Reduction (before coagulation)	+	+				

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp. 28°C.

Nitrite: -3 d. ; ..... d. ; ..... d.

Gas (N): -3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium Lead acetate & Age: 10 day

H_2S: *present*, *absent*. Thio iron Temp. 28°C.

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a Age: 10 day

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (10 °C.): *present*, *absent*. (+-)

Growth at room temperature (28°C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*. (+ - >

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermentative - anaerogenic - Glucose and Sucrose
Nonoxidative-nonfermentative - Lacktose and Xylose

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate-- Positive
Glucose - Positive
Sucrose - Negative
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 19A2
Trypticase-soy agar
2 days

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 2mm

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 2hb (Maize)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus pumilus Studied by Dr. W.B. Bollen Culture No. 19D1

Source Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*)

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28 °C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size 0.65 x 2.5μ Irregular forms:
0.5-0.75 x 1.5-3.5μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

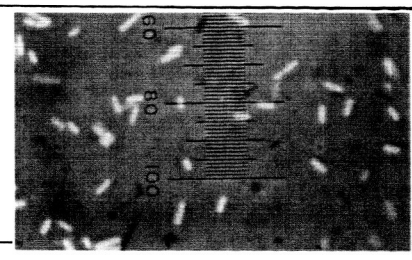
Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin

Sketches



STAINING CHARACTERISTICS

Gram: Age: 24hr. Method: Kopeloff's (modified)

Special stains:

AGAR STROKE Age: 24 hour Temp. 25 °C.

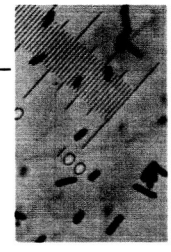
Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: White ; *fluorescent, iridescent, photogenic.*

Gram Stain



24 hr

48 hr

AGAR COLONIES Age: 5 day Temp. 28 °C.

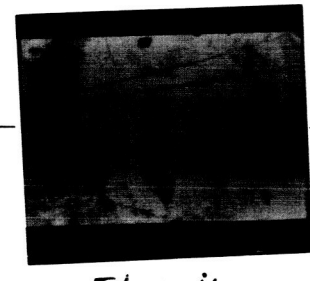
Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*



Flagella

NUTRIENT BROTH Age: 2 day Temp. 25°C.

Surface growth: *none, ring, pellicle, flocculent, membranous.* islands

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 5 day Temp. 25°C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 3ng Yellow maple

Soybean agar slant: 3ca

Fat agar: 2gc Clear to

FERMENTATION Temp. 25 °C.

Medium Nutrient broth: Carbohydrate: 1% Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	=	=	+	=	
Acid in days					
Gas in 2 days	—	—	-	.	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days					
	8	21				
Reaction	=	=				
Acid curd	=	=				
Rennet curd	=	=				
Peptonization	=	+				
Reduction (before coagulation)	+	+				

ACTION ON NITRATES

Medium: 1% KNO3 broth Temp. 28c.

Nitrite: .....d. ; .....d. ; -3.....d.

Gas (N): .....d. ; .....d. ; -3.....d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 da.

Method: Kovac's Temp. 28°C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio iron Age: 10 da. Temp. 28c.

H_2S: *present, absent.*

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present, absent.* ( )

Growth at room temperature (28°C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.* ( )

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a Age: 10 da.

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermentative - anaerogenic - Glucose and Sucrose
Nonoxidative-nonfermentative - Lactose and Xylose

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 19d1
Staphylococcus soy agar
5 day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 3mm

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 2Eb Bamboo

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus subtilis var. niger Studied by Dr. W.B. Bollen Culture No. 1962-2

Source Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28 °C.

Vegetative cells: Age: 24 hrs.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.75 x 2.45μ Irregular forms:
0.75 x 2.0 - 3.0μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to eccentric, terminal, subterminal.*

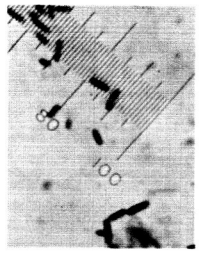
Nigrosin

STAINING CHARACTERISTICS

Gram: + Age: 18hr. Method: Kopeloff's (modified)

Special stains:

24 hr



48 hr

AGAR STROKE Age: 18hr. Temp. 25c.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, folded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: ; *fluorescent, iridescent, photogenic.*

White becoming black in 10 days.
Black water soluble pigment.

Gram stain

AGAR COLONIES Age: 24 hr. Temp. 28 °C.

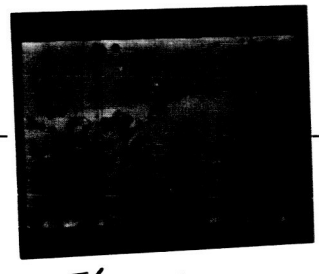
Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*



Flagella

NUTRIENT BROTH Age: 2 day-8 islands Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 2 day Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant:	31e	Camel
Soybean agar slant:	31i	Beaver abundant
Glucose nitrate slant:		scant
Tyrosine agar slant:		Black pigment
Fat agar:	3nl	Dark Brown

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	=	+	−	
Acid in days					
Gas in 2 days	−	−	−	−	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 4	7				
Reaction	−					
Acid curd						
Rennet curd						
Peptonization	+					
Reduction (before coagulation)		+				

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp. 28c.

Nitrite: 3 d. ; ..... d. ; ..... d.

Gas (N): 3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day

Method: Kovac's Temp. 28c.

Indole: *present*, *absent*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate& Thio iron Age: 10 da. Temp. 28c.

H_2S: *present*, *absent*

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a Age: 10 day

Method: Shake tubes Temp. 28c.

Aerobic growth: *absent*, *present, better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present*, *absent*.

Growth at room temperature (28°C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermenter (anaerogenic) Glucose and Sucrose
Nonoxidizer-nonfermenter Lactose and Xylose

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 1962-2
Erythrocase soy agar
24 hour

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 3mm

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3gc St. Sau

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus subtilis Studied by Dr. W. B. Boller Culture No. 19H1

Source Habitat Soil Date 4-2-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticas soy agar Temp. 28 °C.

Vegetative cells: Age: 24 hrs.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.6 x 2.2μ Irregular forms:
0.5-0.75 x 1.5-2.0μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

N: gross

STAINING CHARACTERISTICS

Gram: + - - Age: 24hr. Method Kopeloff's (modified)

Special stains:

24hr

48 hr

AGAR STROKE Age: 24hrs. Temp. 25 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: white ; *fluorescent, iridescent, photogenic.*

Gram stain

AGAR COLONIES Age: 7 days Temp. 25 °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex* center

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 12 days Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 6 days Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 3ne Topaz
Soybean agar slant: 2ca Lt. Ivory
Fat agar: 2ea Lt. wheat

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	+	+	-	
Acid in days					
Gas in 2 days	-	-	-	-	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 4	5	7			
Reaction	-	-	-			
Acid curd	=	=	=			
Rennet curd	=	=	=			
Peptonization	=	=	+			
Reduction (before coagulation)	=	+	+			

ACTION ON NITRATES

Medium 1% KNO_3 broth Temp 28 °C.

Nitrite: -3 d. ; ..... d. ; ..... d.

Gas (N): -3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate & Thio-Iron Age: 10 day Temp. 28°C.

H_2S: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (10 °C.): *present*, *absent*.

Growth at room temperature (28C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*.

RELATION TO FREE OXYGEN - Catalase: Negative

Medium: Dextrose Nutrient a Age: 10 day

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermenter - anaerogenic - Glucose and Sucrose
Nonoxidizer- nonfermenter - Lactose and Xylose

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Negative
Sucrose - Positive
Xylose - Negative

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 1941

Trypticase soy agar 2 Days

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 2mm

2. Shape: Outline- round, oval, irregular, filamentous.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 2ga (St. Lemon Yello

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus subtilis var. niger Studied by Dr. W.B. Boller Culture No. 19H2

Source Habitat Soil Date

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28 °C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.75 x 2.65μ Irregular forms: Filaments
0.73 x 2.9 - 3.5μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

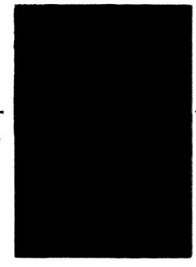
Position: *central to excentric, terminal, subterminal.*

Nigrosin

STAINING CHARACTERISTICS

Gram: + - - Age: 18 Method: Kopeloff's (modified)

Special stains:



24 hrs

48 hrs

AGAR STROKE TS Age: 18 hr. Temp 25 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: White ; *fluorescent, iridescent, photogenic.*

Brown in 10 days (water soluble pigment)

Gram stain

AGAR COLONIES TS Age: 5 da Temp. °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

Flagella

NUTRIENT BROTH Age: 2 days Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 2 days Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant:	3ie	Camel
Soybean agar slant:	31g	Adobe Brown
Glucose nitrate slant:		White Abundant growth
Tyrosine agar slant:		Not blackened - slightly darkened.
Fat agar:	3nl	Dark Brown

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth
Carbohydrate: 1 %
Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	-	+	-	
Acid in days					
Gas in 2 days	-	-	-	-	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus

	Days					
	3	4				
Reaction	=					
Acid curd						
Rennet curd						
Peptonization		+				
Reduction (before coagulation)	+					

ACTION ON NITRATES

Medium: 1%KNO3 broth Temp. 28°C.

Nitrite: +3d. ; ....d. ; ....d.

Gas (N): -3d. ; ....d. ; ....d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 days

Method: Kovac's Temp. 28°C.

Indole: *present, absent,*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio Iron Age: 10 day

H_2S: *present, absent.* Temp. 28°C.

RELATION TO FREE OXYGEN- Catalase: Positive-

Medium: Dextrose Nutrient a Age: 10da. Negative

Method: Shake tubes Temp. 28°C.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present, absent.* (+-)

Growth at room temperature (28°C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Fermenter - anaerogenic (Glucose and Sucrose)
Nonoxidizer-nonfermenter (Lactose & Xylose)

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Positive

Cultural Characteristics of Bacterial Colonies

Culture No. 1942
Trypticase soy agar
5 day

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 6 m.m.

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3gc St. Ivory

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus polymyxa Studied by Dr. W.B. Bollen Culture No. 191

Source White Mountain Habitat Soil Date

Descriptions (*Underscore required terms.*)	Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28 °C.

Vegetative cells: Age: 24hrs

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.8 x 2.9μ / 0.75-1 x 1.5-4.0μ Irregular forms:

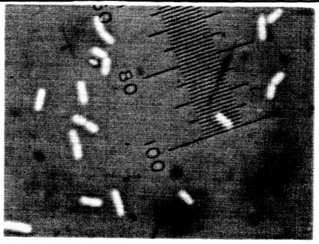
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin



STAINING CHARACTERISTICS

Gram: + − Age: 24hr Method: Kopeloff (modified)

Special stains:

Gram stain

24 hr. 48 hr

AGAR STROKE Age: 24hrs. Temp. 28 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: Ivory ; *fluorescent, iridescent, photogenic.*

AGAR COLONIES Age: 48 hours Temp. 28 °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

Flagella

NUTRIENT BROTH Age: 24hrs. Temp. 28 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 5 days Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: 10 days Temp. 28 °C.

Potato slant:	2db	Ivory
Soybean agar slant:	3ec	Bisque (abundant)
Glucose nitrate slant:		White (abundant)
Fat agar:		White
Tyrosine agar slant:		No pigment.

FERMENTATION Temp. 28 °C.

Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	+	⊕	+	
Acid in days					
Gas in 2 days	+	+−	+	+	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator:	Days					
Litmus	3					30
Reaction						Acid
Acid curd	+ (coag-s' whey sep.)					+
Rennet curd						
Peptonization						+
Reduction (before coagulation)	+					+

ACTION ON NITRATES

Medium: 1%KNO_3 broth Temp. 28 °C.

Nitrite: -3 d. ; .... d. ; .... d.

Gas (N): -3 d. ; .... d. ; .... d.

INDOLE PRODUCTION

Medium: Tryptophan Age: 10 days

Method: Kovac's Temp. 30 °C.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio-iron Age: 10 days

H_2S: *present*, *absent*. Temp. 30 °C.

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient A Age: 10 days

Method: Shake Tubes Temp. 30 C.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present*, *absent*.

Growth at room temperature (28°C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 45° C.: *present*, *absent*.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Negative

Metabolism: Fermenter - aerogenic

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Negative
10% - Negative

Reduces Brom Thymol Blue.

Cultural Characteristics of Bacterial Colonies

Culture No. 19 I
Trypticase soy agar
2 days

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 3

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 1ca (pale yellow)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus polymyxa * Studied by Dr. W.B. Bollen Culture No. 118D

Source Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp 28 °C.

Vegetative cells: Age: 24Hrs.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 1.1 x 6.0μ Irregular forms:

1.0-1.5 x 4.0-8.0μ

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin

STAINING CHARACTERISTICS

Gram: + – – Age: 18hr Method: Kopeloff's (modified)

Special stains:

AGAR STROKE Age: 18 hr. Temp. 25 °C.

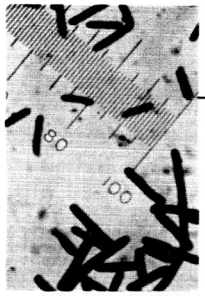
Amount of growth: *scanty, moderate, abundant.*

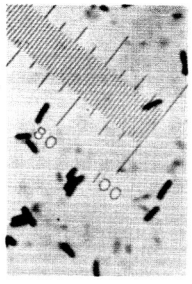
Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: White ; *fluorescent, iridescent, photogenic.*

Gram stain





24 hr 48 hr

AGAR COLONIES Age: 10 day Temp. 28 °C.

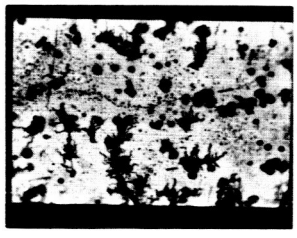
Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*



Flagella

NUTRIENT BROTH Age: 8 day Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 10 day Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant:	2db	Ivory
Soybean agar slant:		White abundant
Glucose nitrate slant:		scant
Tyrosine agar slant:		Black pigment
Fat agar:	2ec	Biscuit

PERMENTATION Temp. 25°C.

Medium: Nutrient broth
Carbohydrate: 1 %
Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	=	+	–	
Acid in days					
Gas in 2 days	+		+		
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus

	Days					
	5	35				
Reaction	+					
Acid curd	±	+ coag.t (whey stp)				
Rennet curd	=					
Peptonization	=					
Reduction (before coagulation)	+					

+ = acid

* Varies from typical B. polymyxa in casein and gelatin hydrolysis (negative), and salt tolerances (2% negative).

ACTION ON NITRATES

Medium: 1%KNO_3 broth Temp. 28°C.

Nitrite: - 3 d. ; ..... d. ; ..... d.

Gas (N): - 3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate & Age: 10 da.

H_2S: *present, absent.* Thio iron Temp. 28°C.

TEMPERATURE RELATIONS

Growth in refrigerator (°C.): *present, absent.*

Growth at room temperature (°C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a. Age: 10 day

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Negative
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Negative
Starch Hydrolysis: Negative
Urea Hydrolysis: Negative

NH_4 from Peptone: Negative

Metabolism: Fermentative, aerogenic

Acetyl methyl carbinol: Positive

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Negative
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Negative
7% - Negative
10% - Negative

Reduces Brom Cresol Purple and Brom Thymol Blue.

Cultural Characteristics of Bacterial Colonies

Culture No. 118d
Bryphrase soy agar
10 days.

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 1mm.

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 1½ca Cream

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus circulans * Studied by Dr. W.B. Bollen Culture No. 122A

Source Habitat Soil Date 4-8-65

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28 °C.

Vegetative cells: Age: 24hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 0.65 x 2.0µ Irregular forms:

0.3-0.75 x 10-25µ

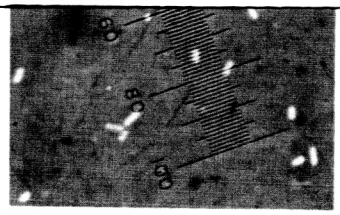
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin



STAINING CHARACTERISTICS

Gram: + − − Age: 18hr. Method: Kopeloff's (modified)

Special stains:

AGAR STROKE Age: 18 hr. Temp. 25 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: White ; *fluorescent, iridescent, photogenic.*

Gram Stain

24 hr

48 hr

AGAR COLONIES Age: 2 day Temp. 28 °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 8 day Temp. 25 °C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 2 day Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant:	3ba	Pearl
Soybean agar slant:		White
Glucose nitrate slant:		scant
Tyrosine agar slant:		No pigment
Fat agar:	2gc	Covert Tan

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	+	+	+	
Acid in days					
Gas in 2 days	−	−	−	−	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 2	13				
Reaction	+					
Acid curd		+	(coag & whey sep.)			
Rennet curd						
Peptonization						
Reduction (before coagulation)	+					

* small variant.

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp. 28°C.

Nitrite: -3d. ; ....d. ; ....d.

Gas (N): -3d. ; ....d. ; ....d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead acetate & Thio iron Age: 10 day

H_2S: *present, absent.* Temp. 28°C.

TEMPERATURE RELATIONS

Growth in refrigerator (°C.): *present, absent.*

Growth at room temperature (°C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose Nutrient a Age: 10 day

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Positive
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Negative

Metabolism: Fermenter - anaerogenic

Acetyl methyl carbinol: Negative

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Positive
Glucose - Negative
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Negative

Crystalline Dextrins: Observed

Salt tolerances: 2% - Positive
7% - Negative
10% - Negative

Cultural Characteristics of Bacterial Colonies

Culture No. 122A
Trypticase soy agar
2 days.

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 1 m.m.

2. Shape: Outline- round, oval, irregular, filamentous.
Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 1½ ca (cream)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color

Name of organism Bacillus circulans Studied by Dr. W.B. Bollen Culture No. 126

Source Habitat Soil Date

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy Temp. 28 °C.

Vegetative cells: Age: 24hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: + Flagella: Peritrichous

Size: 1x4 - 5.0μ Irregular forms:

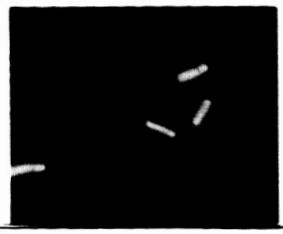
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

Nigrosin



STAINING CHARACTERISTICS

Gram: + − − Age: 18hr Method: Kopeloff (modified)

Special stains:

AGAR STROKE Age: 18 hrs. Temp. 25 °C.

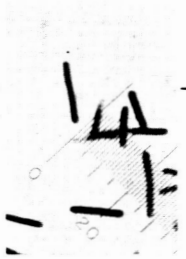
Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: White ; *fluorescent, iridescent, photogenic.*

Gram Stain



24hr

48 hr

AGAR COLONIES Age: Temp. °C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 8 days Temp. 25°C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: Temp. °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant: 31g Nude tan

Soybean agar slant: Grey-white (scant)

Glucose nitrate slant: Scant

Fat agar: 3nl Dark Brown (abundant) medium darkened

Tyrosine agar slant: No pigment

Glucose nutrient agar slant: growth as good or better than nutrient agar

FERMENTATION Temp. 28 °C.

Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	+	+	+	
Acid in days					
Gas in 2 days	−	−	−	−	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 1		20			
Reaction	Alk					
Acid curd			+ (coag. & whey sep.)			
Rennet curd						
Peptonization						
Reduction (before coagulation)	+					

ACTION ON NITRATES

Medium: 1%KNO3 broth Temp. 28°C.
Nitrite: -+3 d. ; ..... d. ; ..... d.
Gas (N): -+3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 days
Method: Kovac's Temp. 28°C.
Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio-iron Age: 10days
H_2S: *present*, *absent*. Temp 28 °C.

RELATION TO FREE OXYGEN - Catalase: Positive - Neg

Medium: Dextrose-Nutrient A. Age: 10 days
Method: Shake Tubes Temp. 28°C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

Anaerobic growth: *present*, *absent*.

TEMPERATURE RELATIONS

Growth in refrigerator (10 °C.): *present*, *absent*. (+-)
Growth at room temperature (28 °C.): *present*, *absent*.
Growth at 37° C.: *present*, *absent*.
Growth at 50° C.: *present*, *absent*.

Pasteurization survival, 80°C. 10 minutes: Positive

ADDITIONAL TESTS

Casein Hydrolysis: Positive
Fat Hydrolysis: Negative
Gelatin Hydrolysis: Positive
Starch Hydrolysis: Positive
Urea Hydrolysis: Negative

NH_4 from Peptone: Negative

Metabolism: Fermenter - anaerogenic

Acetyl methyl carbinol: Negative

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources: Citrate - Negative
Glucose - Positive
Sucrose - Positive
Xylose - Positive

Methylene blue reduction: Positive 1day: Negative 5 days.

Crystalline Dextrins: Not observed

Salt tolerances: 2% - Positive
7% - Positive
10% - Negative

Cultural Characteristics of Bacterial Colonies

Culture No. 126
Trypticase soy agar
2 days.

I. Surface Colonies

a. Macroscopic appearance.

1. Size, mm 1 mm

2. Shape: Outline- round, oval, irregular, filamentous. Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate. Topography- smooth, rough, wrinkled, contoured, striated. Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 1ea (pale yellow)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, cremate, erose, ciliate, rhizoid.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

II. Deep colonies

a. Size, mm

b. Shape

c. Color