PROGRESS REPORT

MICROORGANISM STUDY, CIT CONTRACT NO. 950783

Jet Propulsion Laboratory

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# Systematic Description and Key to Isolants from Little Lake Volcanic Area, California

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The following described bacteria are isolants from soils of the Little Lake Volcanic Area in California. The individual isolants are separated into groups by a Dichotomous Key. The Descriptive Charts are arranged in these groups. In addition to this dichotomous key is a list of the isolants and species designations in the order of their code numbers.

Of the 19 cultures received, isolant 196Aa contained two different bacterial species, and isolant 196Ab exhibited no growth upon the original slant. Of these 19 isolants, 73.7% are bacteria, 10.5% are actinomycetes, 10.5% are yeasts, and 5.3% are molds.

Of the bacteria 50.0% are species of the genus Bacillus, 42.9% are soil diphtheroids, and 7.1% are species of Micrococcus.

The pictures in the following report are all 1000x magnification. The nigrosin stains were prepared from 24 hour cultures. Methylene blue spore stains were from 12 hour cultures.

## LIST OF ISOLANTS AND SPECIES DESIGNATIONS

| Code Number | Species Designation | Page |
|---|---|---|
| 196Aa-1 | *Bacillus megaterium*. | 11 |
| *196Aa-2 | "Soil diphtheroid". | 3 |
| 196Ab | No growth upon original slant. | |
| 196Ac | *Bacillus megaterium*. | 13 |
| 197Aa | "Soil diphtheroid." | 5 |
| 197Ab | *Bacillus megaterium*. | 15 |
| 197Bc | *Bacillus megaterium*. | 17 |
| 300Aa | *Bacillus megaterium*. | 19 |
| *300Ab | "Soil diphtheroid." | |
| 300Bc | *Micrococcus* sp. | 1 |
| **300Bd | Yeast. | |
| 300Ae | *Bacillus megaterium*. | 21 |
| *301Aa | "Soil diphtheroid." | |
| 301Ab | Actinomycete. | |
| 302Aa | Mold. | |
| 302Bb | Actinomycete. | |
| 303Ba | "Soil diphtheroid." | 7 |
| **303Bb | Yeast. | |
| 304Aa | *Bacillus subtilis* var. *niger*. | 23 |
| 304Bc | "Soil diphtheroid." | 9 |

# DICHOTOMOUS KEY

I. Molds.

302Aa

II. Yeasts.**

300Bd
303Bb

III. Actinomycetes.

301Ab
302Bb

IV. Bacteria.

A. Isolants failing to grow upon original transfer.

1. No visible growth on original slant.

196Ab

2. Growth on original slant; soil diphtheroid.

301Aa

B. Isolants failing to grow on subsequent transfers.

196Aa-2 "Soil diphtheroid."
300Ab "Soil diphtheroid."

C. Growing isolants; Gram-positive.

1. Do not survive pasteurization.

a. Cocci.

| | | |
|---|---|---|
| 300Bc | *Micrococcus* sp. | 1 |

b. Non-sporulating rods.

| | | |
|---|---|---|
| *196Aa-2 | "Soil diphtheroid." | 3 |
| 197Aa | "Soil diphtheroid." | 5 |
| *300Ab | "Soil diphtheroid." | |
| 303Ba | "Soil diphtheroid." | 7 |
| 304Bc | "Soil diphtheroid." | 9 |

2. Survive pasteurization.

The following key for isolants surviving pasteurization is in accord with the arrangement of:

(1) Smith, Nathan R., Ruth E. Gordon, and Francis E. Clark. 1952. Aerobic Sporeforming Bacteria. Agriculture Monograph No. 16. U.S. Department of Agriculture.

and (2) Breed, Robert S., E. G. D. Murray, and Nathan R. Smith. 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. Baltimore. The Williams and Wilkins Company. 1094 pp.

I. Sporangia not definitely swollen. Spores ellipsoidal to cylindrical, central to terminal. Spore wall thin and not easily stained. Gram-positive.

A. Diameter of vegetative rods is 0.9 micron or more.

1. Acetylmethylcarbinol not produced.
Acid from mannitol with ammonium salts as sole source of nitrogen.

*Bacillus megaterium*.

| | |
|---|---|
| 196Aa-1 | 11 |
| 196Ac | 13 |
| 197Ab | 15 |
| 197Bc | 17 |
| 300Aa | 19 |
| 300Ac | 21 |

B. Diameter of vegetative rods less than 0.9 micron.

1. Growth on glucose-nutrient agar as good or better than on nutrient agar; good growth on soybean agar.

a. Growth in 5 percent NaCl broth; strong hydrolysis of gelatin.

(1) Starch hydrolyzed; nitrates reduced to nitrites.

(a) Scant if any growth under anaerobic conditions, no gas from nitrates under alkaline anaerobiosis.

(aa) Black pigment on tyrosine mediums only.

*Bacillus subtilis* var. *niger*

304Aa 23

* Died upon further transfer.
** See page 25 of this report.

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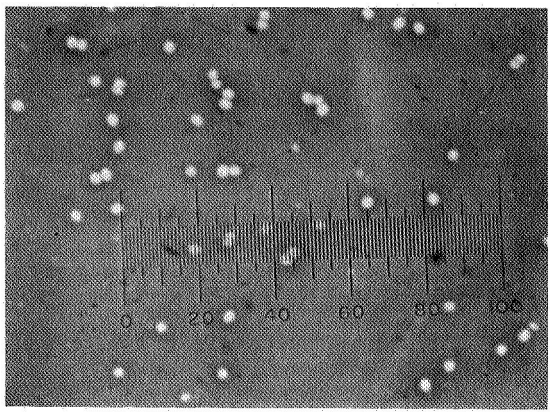
# Descriptive Chart

| 300Bc | Trypticase Soy Agar | Little Lake, California |
|---|---|---|
| (code number) | (medium) | (source) |
| Micrococcus sp.* | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*




MORPHOLOGY:
Form: *rods, ends*_____,
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* – 1.13 x 1.29μ.
*range* – 0.96-1.29 x 1.06-1.41μ.
Irregular forms:

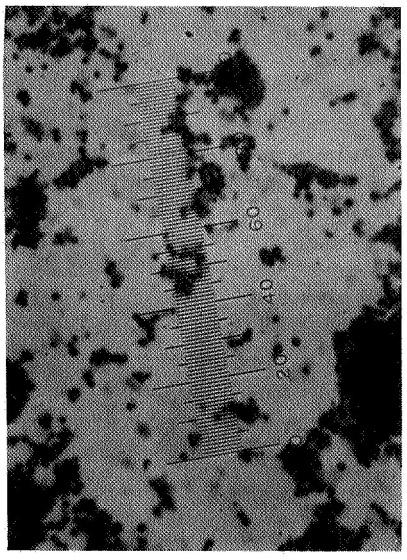
NIGROSIN:

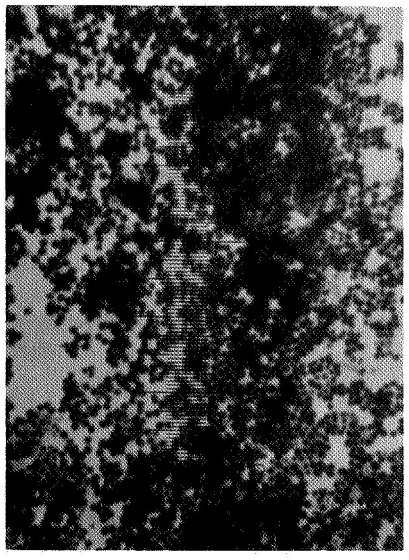
GRAM REACTION:
18 hrs: 50% POSITIVE.
24 hrs: 80% POSITIVE.
48 hrs: 100% NEGATIVE.

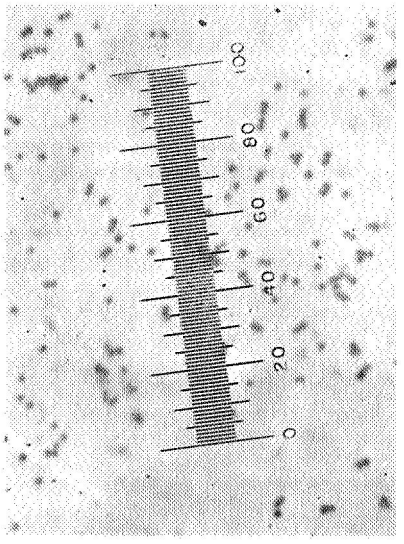
PASTEURIZATION SURVIVAL (85°C, 10 minutes): NEGATIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average –
range –

MOTILITY: age 1 DA. NEGATIVE.
Flagella:

OTHER STAINS:
~~Acid fast~~ 18; 24; 48 hr. GRAM:






* resembling M. roseus and M. rubens.

300 BC

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 8 DA.

Macroscopic

Size: 6 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | ORANGE | 4 la |
| Potato slant | NO GROWTH | |
| — | | |
| — | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 18 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: GOOD.

Fat agar:

Glucose-nitrate agar: NO GROWTH.

GLUCOSE-NUTRIENT AGAR: MODERATE.

NUTRIENT AGAR: EXCELLENT.

ANAEROBIC NITRATE BROTH: NEGATIVE.

ANAEROBIC GLUCOSE BROTH: GROWTH. pH 4.2.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at ~~10~~ 5.5°C. +, 20°C. +, 28°C. +, 37° +, 45°C. +, 55°C. −.

SOLE CARBON SOURCE: age 7 DA.

| | |
|---|---|
| Glucose: *positive, negative.* | 7 DA. |
| Sucrose: *positive, negative.* | 7 DA. |
| Xylose: *positive, negative.* | 7 DA. |
| Citrate: *positive, negative.* | 7 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 7 DA. |
| MANNITOL: POSITIVE. | 7 DA. |
| LACTOSE: POSITIVE. | 7 DA. |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$ +, $NH_4^+$ ___, *gas* ___, *negative.* | 1 DA. |
| Methylene blue: *positive, negative.* | 16 DA. |
| Selenite: *positive, negative.* | ___ |
| Tellurite: *positive, negative.* | ___ |
| — | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Sucrose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Lactose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Xylose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Mannitol: *acid* +, *alkaline, neutral, gas.* | 7 DA. |
| — | |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | 12 DA. |
| Casein: *positive, negative.* | 12 DA. |
| Fat: *positive, negative.* | 7 DA. |
| Starch: *positive, negative.* | 7 DA. |
| Cellulose: *positive, negative.* | ___ |
| Urea: *positive, negative.* | ___ |

TOLERANCES:

| | |
|---|---|
| Salt: 2%—*positive, negative.* | 7 DA. |
| 7%—*positive, negative.* | |
| 10%—*positive, negative.* | |
| pH: *acid* ___, *alkaline* ___. | ___ |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 20 DA. |
| Curd: *acid, alkaline, absent, gas.* | 20 DA. |
| Peptionization: *positive, negative.* | 20 DA. |
| Reduction: *positive, negative.* | 20 DA. |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from ___: *positive, negative.* | ___ |
| $NH_4^+$ from peptone: *positive, negative.* | 7 DA. |
| Acetylmethylcarbinol: *positive, negative.* | 15 DA. |
| Indol: *positive, negative.* | 23 DA. |
| Methyl red: *positive, negative.* | 15 DA. |

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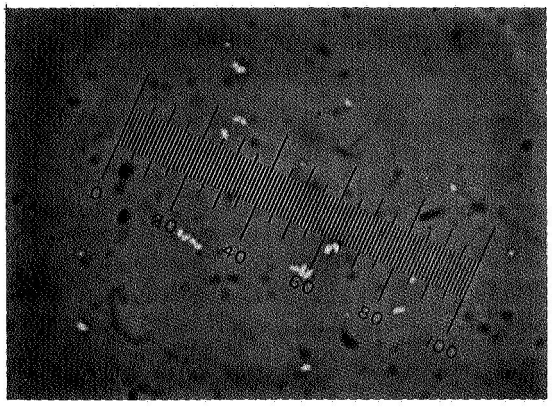
# Descriptive Chart

| 196Aa - 2 | Trypticase Soy Agar | Little Lake, California |
|---|---|---|
| (code number) | (medium) | (source) |
| "soil diphtheroid" | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*



NIGROSIN: 24 HR. 1000X

MORPHOLOGY:
Form: *rods*, *ends* POINTED-ROUND, *filaments, cocci, spirals, branching* _____.
Size: *average* –
*range* –
Irregular forms:

GRAM REACTION:
18 hrs:
24 hrs:
48 hrs:

PASTEURIZATION SURVIVAL (85°C, 10 minutes): NEGATIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average –
range –

MOTILITY: age _____.
Flagella:

OTHER STAINS:
Acid fast:
Capsule:
Glycogen:
Crystalline dextrins:
Fat globules:
Metachromatic granules:

THIS CULTURE GREW ON THE FIRST FEW TRANSFERS, BUT ON SUBSEQUENT TRANSFERS DIED.

196 Aa-2

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 8 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 8 DA.

Macroscopic

Size: 0.5 mm.

Shape: *filamentous, irregular, oval, punctiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | CANARY YELLOW | 180 |
| Potato slant | | |
| — | | |
| — | | |

NUTRIENT BROTH: age _____.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling _____.

GELATIN STAB: age _____.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: MODERATE GROWTH.

Fat agar:

Glucose-nitrate agar:

GLUCOSE-NUTRIENT AGAR GROWTH BETTER THAN NUTRIENT AGAR GROWTH.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age _____.

Growth at 10°C.____, 20°C. ____, 28°C.____, 37°____, 45°C.____, 55°C.____.

SOLE CARBON SOURCE: age _____.

Glucose: *positive, negative.* _____

Sucrose: *positive, negative.* _____

Xylose: *positive, negative.* _____

Citrate: *positive, negative.* 7 DA

$NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* 7 DA.

—

REDUCTIONS:

Nitrate: $NO_2^-$____, $NH_4^+$____, *gas*____, *negative.* _____

Methylene blue: *positive, negative.* _____

Selenite: *positive, negative.* _____

Tellurite: *positive, negative.* _____

—

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid*____, *alkaline*____, *neutral, gas.* _____

Sucrose: *acid*____, *alkaline*____, *neutral, gas.* _____

Lactose: *acid*____, *alkaline*____, *neutral, gas.* _____

Xylose: *acid*____, *alkaline*____, *neutral, gas.* _____

Mannitol: *acid*____, *alkaline, neutral, gas.* _____

—

HYDROLYSIS:

Gelatin: *positive, negative.* _____

Casein: *positive, negative.* 7 DA.

Fat: *positive, negative.* _____

Starch: *positive, negative.* _____

Cellulose: *positive, negative.* _____

Urea: *positive, negative.* _____

TOLERANCES:

Salt: 2%—*positive, negative.* _____

7%—*positive, negative.*

10%—*positive, negative.*

pH: *acid*_____, *alkaline*_____. _____

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.* _____

Curd: *acid, alkaline, absent, gas.* _____

Peptionization: *positive, negative.* _____

Reduction: *positive, negative.* _____

OTHER REACTIONS:

$H_2S$ from _____: *positive, negative.* _____

$NH_4^+$ from peptone: *positive, negative.* _____

Acetylmethylcarbinol: *positive, negative.* _____

Indol: *positive, negative.* _____

Methyl red: *positive, negative.* _____

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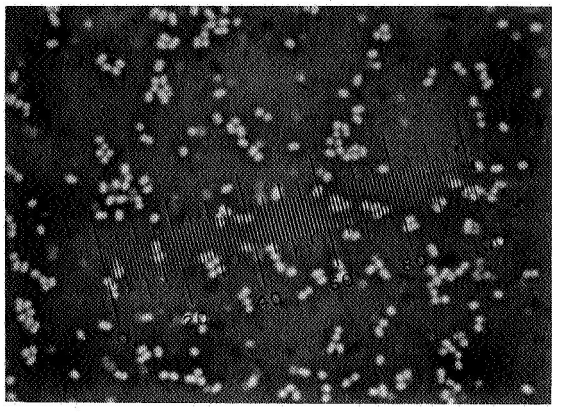
# Descriptive Chart

| | | |
|---|---|---|
| 197Aa | Trypticase Soy Agar | Little Lake, California |
| (code number) | (medium) | (source) |
| "soil diphtheroid" | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*



NIGROSIN:

MORPHOLOGY:
Form: *rods*, *ends* ROUND,
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* – 0.88 x 1.22 μ.
*range* – 0.74 - 1.06 x 0.80 - 1.61 μ.
Irregular forms:

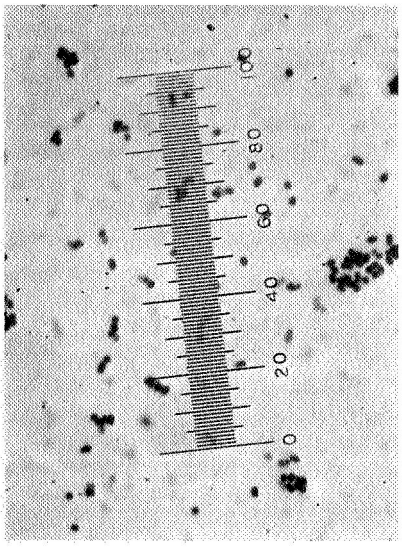
GRAM REACTION:
18 hrs: 100 % POSITIVE.
24 hrs: 100 % POSITIVE.
48 hrs: 100 % POSITIVE.

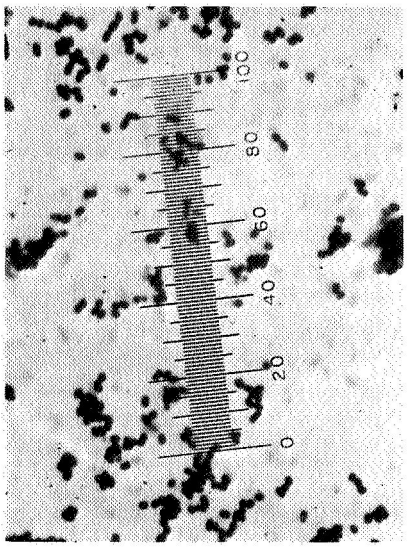
PASTEURIZATION SURVIVAL (85°C, 10 minutes): NEGATIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average –
range –

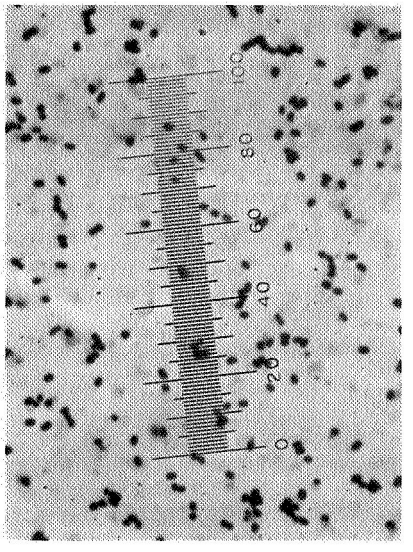
MOTILITY: age 1 DA. NEGATIVE.
Flagella:

OTHER STAINS:
~~Acid fast~~:
Capsule:

18: 24: 48 GRAM:







197AA

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 2 DA.

Macroscopic

Size: 2 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | SUN ORANGE | 5la |
| Potato slant | ORANGE | 5la |
| — | | |
| — | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 12 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: NO GROWTH.

Fat agar:

Glucose-nitrate agar: FAINT.

GLUCOSE-NUTRIENT AGAR: SCANT.

NUTRIENT AGAR: GOOD.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: SLIGHT GROWTH pH 7.2.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at ~~10~~ 4.5°C. +, 20°C. +, 28°C. +, 37° +, 45°C. +, 55°C. +.

SOLE CARBON SOURCE: age 7 DA.

Glucose: *positive, negative.* 7 DA.

Sucrose: *positive, negative.* 7 DA.

Xylose: *positive, negative.* 7 DA.

Citrate: *positive, negative.* 7 DA.

$NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* 7 DA

MANNITOL: POSITIVE. 7 DA.

LACTOSE: POSITIVE. 7 DA.

REDUCTIONS:

Nitrate: $NO_2^-$ ____, $NH_4^+$ ____, *gas* ____, *negative.* 11 DA.

Methylene blue: *positive, negative.* 20 DA.

Selenite: *positive, negative.* ____

Tellurite: *positive, negative.* ____

—

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid* ____, *alkaline* ____, *neutral, gas.* 23 DA.

Sucrose: *acid* ____, *alkaline* +, *neutral, gas.* 7 DA.

Lactose: *acid* ____, *alkaline* +, *neutral, gas.* 7 DA.

Xylose: *acid* ____, *alkaline* +, *neutral, gas.* 7 DA.

Mannitol: *acid* ____, *alkaline, neutral, gas.* 7 DA.

—

HYDROLYSIS:

Gelatin: *positive, negative.* ____

Casein: *positive, negative.* 7 DA.

Fat: *positive, negative.* 7 DA.

Starch: *positive, negative.* 7 DA.

Cellulose: *positive, negative.* ____

Urea: *positive, negative.* ____

TOLERANCES:

Salt: 2%—*positive, negative.* 7 DA.

7%—*positive, negative.*

10%—*positive, negative.*

pH: *acid* ____, *alkaline* ____. ____

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.* 20 DA.

Curd: *acid, alkaline, absent, gas.* 20 DA.

Peptionization: *positive, negative.* 20 DA.

Reduction: *positive, negative.* 20 DA.

OTHER REACTIONS:

$H_2S$ from ____: *positive, negative.* ____

$NH_4^+$ from peptone: *positive, negative.* 7 DA.

Acetylmethylcarbinol: *positive, negative.* 15 DA.

Indol: *positive, negative.* 23 DA.

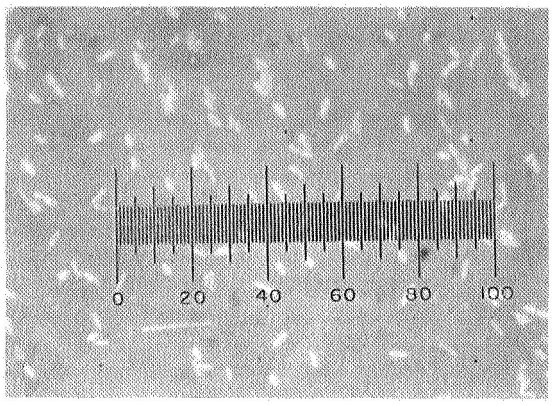
Methyl red: *positive, negative.* 15 DA.

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
(JPL–NASA)

# Descriptive Chart

| | | |
|---|---|---|
| 303Ba | Trypticase Soy Agar | Little Lake, California. |
| (code number) | (medium) | (source) |
| "Soil diphtheroid" | 28°C | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*




NIGROSIN:

MORPHOLOGY:

Form: *rods*, *ends* POINTED,
*filaments, cocci, spirals,*
*branching* _____.

Size: *average* – 0.70 x 2.18 μ.
*range* – 0.55 – 0.94 x 1.88 – 2.60 μ.

Irregular forms:

GRAM REACTION:

18 hrs: 100 % POSITIVE.
24 hrs: 100 % POSITIVE.
48 hrs: 100 % POSITIVE.

PASTEURIZATION SURVIVAL (85°C, 10 minutes): NEGATIVE.

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

Endospores: *swollen, not swollen,.*

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

Shape: *spherical, ellipsoidal, cylindrical, oval.*

size: average –
range –

MOTILITY: age 1 DA. NEGATIVE.

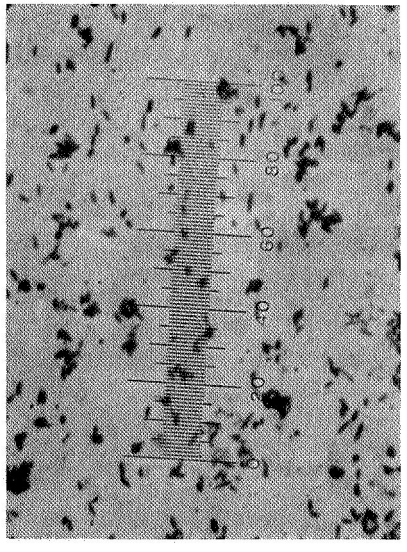
Flagella:

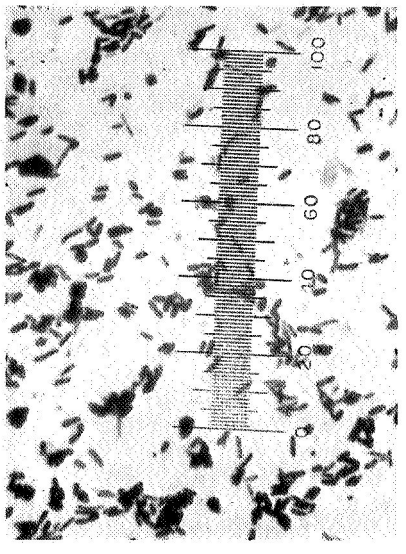
OTHER STAINS:

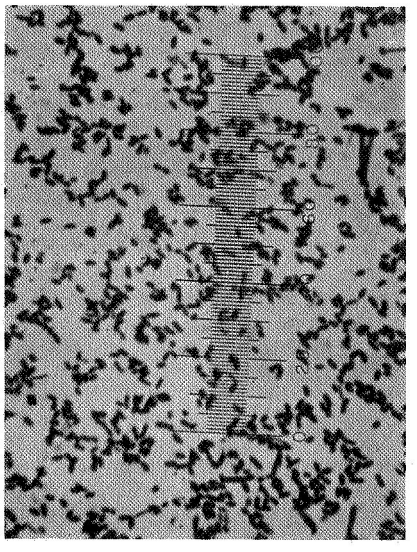
~~Acid fast~~

Granules:

18; 24; 48 hr. GRAM:







303BA

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 5 DA

Macroscopic

Size: 3 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | LT. MELLON YELLOW | 3ea |
| Potato slant | LT. MELLON YELLOW | 3ea |
| – | | |
| – | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling $H_2S$.

GELATIN STAB: age 18 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: FAIR.

Fat agar:

Glucose-nitrate agar: FAIR.

GLUCOSE-NUTRIENT AGAR: GOOD.

NUTRIENT AGAR: MODERATE.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: SLIGHT GROWTH.

–

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at 5°C. –, 20°C. +, 28°C. +, 37° +, 45°C. –, 55°C. –.

SOLE CARBON SOURCE: age _____.

| | |
|---|---|
| Glucose: *positive, negative.* | 7 DA. |
| Sucrose: *positive, negative.* | 7 DA. |
| Xylose: *positive, negative.* | 7 DA. |
| Citrate: *positive, negative.* | 7 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 7 DA. |
| MANNITOL: NEGATIVE. | 7 DA. |
| LACTOSE: NEGATIVE. | 7 DA. |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$ +, $NH_4^+$ ____, *gas* ____, *negative.* | 11 DA. |
| Methylene blue: *positive, negative.* | 1 DA. |
| Selenite: *positive, negative.* | |
| Tellurite: *positive, negative.* | |
| – | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid* +, *alkaline* ____, *neutral, gas.* | 7 DA. |
| Sucrose: *acid* ____, *alkaline* +, *neutral, gas.* | 7 DA. |
| Lactose: *acid* ____, *alkaline* +, *neutral, gas.* | 7 DA. |
| Xylose: *acid* +, *alkaline* ____, *neutral, gas.* | 7 DA. |
| Mannitol: *acid* ____, *alkaline, neutral, gas.* | 7 DA. |
| – | |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | 7 DA. |
| Casein: *positive, negative.* | 12 DA. |
| Fat: *positive, negative.* | 7 DA. |
| Starch: *positive, negative.* | 7 DA. |
| Cellulose: *positive, negative.* | |
| Urea: *positive, negative.* | |

TOLERANCES:

| | |
|---|---|
| Salt: 2%—*positive, negative.* | 7 DA. |
| 7%—*positive, negative.* | |
| 10%—*positive, negative.* | |
| pH: *acid* _____, *alkaline* _____. | |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 20 DA. |
| Curd: *acid, alkaline, absent, gas.* | 20 DA. |
| Peptionization: *positive, negative.* | 20 DA. |
| Reduction: *positive, negative.* | 20 DA |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from _____: *positive, negative.* | |
| $NH_4^+$ from peptone: *positive, negative.* | 7 DA. |
| Acetylmethylcarbinol: *positive, negative.* | 18 DA. |
| Indol: *positive, negative.* | 23 DA. |
| Methyl red: *positive, negative.* | 18 DA. |

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
(JPL–NASA)

# Descriptive Chart

| | | |
|---|---|---|
| 304Bc | Trypticase Soy Agar | Little Lake, California |
| (code number) | (medium) | (source) |
| "soil diphtheroid" | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*

MORPHOLOGY:
Form: *rods, ends* POINTED-ROUND
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* –
*range* –
Irregular forms:

GRAM REACTION:
18 hrs:
24 hrs: 100% POSITIVE.
48 hrs: 100% POSITIVE.

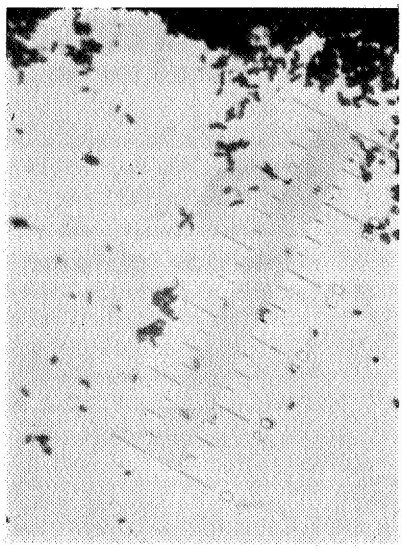
NIGROSIN:

PASTEURIZATION SURVIVAL (85°C, 10 minutes): NEGATIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average –
range –

MOTILITY: age _____.
Flagella:

OTHER STAINS:
Acid fast:
Capsule:
Glycogen:
Crystalline dextrins:
Fat globules:
Metachromatic granules:

24:48 HR. GRAM:



304 Bc

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 22 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 22 DA.

Macroscopic

Size: 3 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | BRITE GOLD | 2pc |
| Potato slant | | |
| – | | |
| – | | |

NUTRIENT BROTH: age _____.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling _____.

GELATIN STAB: age _____.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: ABUNDANT GROWTH.

Fat agar:

Glucose-nitrate agar: NO GROWTH.

GLUCOSE NUTRIENT AGAR GROWTH BETTER THAN NUTRIENT AGAR GROWTH.

ANAEROBIC NITRATE BROTH: NO GAS.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age _____.

Growth at 10°C.____, 20°C. ____, 28°C.____, 37°____, 45°C.____, 55°C.____.

SOLE CARBON SOURCE: age _____.

| | |
|---|---|
| Glucose: *positive, negative.* | |
| Sucrose: *positive, negative.* | |
| Xylose: *positive, negative.* | |
| Citrate: *positive, negative.* | 2 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 2 DA |
| – | |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$____, $NH_4^+$____, *gas*____, *negative.* | |
| Methylene blue: *positive, negative.* | |
| Selenite: *positive, negative.* | 7 DA. |
| Tellurite: *positive, negative.* | |
| – | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid*____, *alkaline*____, *neutral, gas.* | |
| Sucrose: *acid*____, *alkaline*____, *neutral, gas.* | |
| Lactose: *acid*____, *alkaline*____, *neutral, gas.* | |
| Xylose: *acid*____, *alkaline*____, *neutral, gas.* | |
| Mannitol: *acid*____, *alkaline, neutral, gas.* | |
| – | |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | 6 DA. |
| Casein: *positive, negative.* | |
| Fat: *positive, negative.* | |
| Starch: *positive, negative.* | |
| Cellulose: *positive, negative.* | |
| Urea: *positive, negative.* | |

TOLERANCES:

| | |
|---|---|
| Salt: 2%–*positive, negative.* | 2 DA. |
| 7%–*positive, negative.* | |
| 10%–*positive, negative.* | |
| pH: *acid*_____, *alkaline*_____. | |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 7 DA. |
| Curd: *acid, alkaline, absent, gas.* | |
| Peptionization: *positive, negative.* | |
| Reduction: *positive, negative.* | 14 DA. |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from _____: *positive, negative.* | |
| $NH_4^+$ from peptone: *positive, negative.* | |
| Acetylmethylcarbinol: *positive, negative.* | |
| Indol: *positive, negative.* | |
| Methyl red: *positive, negative.* | |

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
(JPL–NASA)

# Descriptive Chart

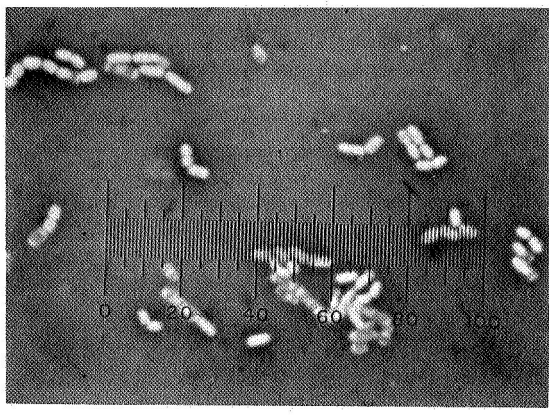
| 196Aa | Trypticase Soy Agar | Little Lake, California |
|---|---|---|
| (code number) | (medium) | (source) |
| Bacillus megaterium. | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*

MORPHOLOGY:
Form: *rods, ends* ROUND,
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* – 1.13 x 2.92 μ.
*range* – 1.12 - 1.26 x 2.40 - 5.22 μ.
Irregular forms:

GRAM REACTION:
18 hrs: 100 % POSITIVE.
24 hrs: 100 % POSITIVE.
48 hrs: 100 % POSITIVE.

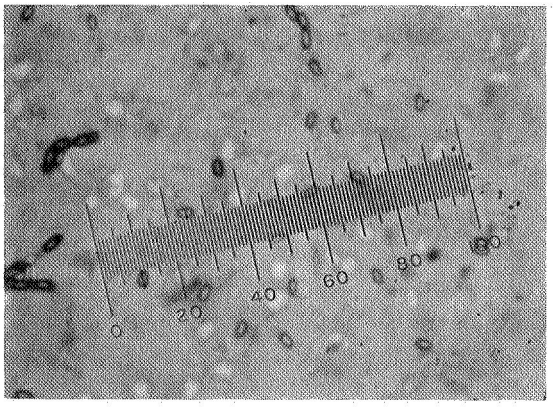
NIGROSIN:



PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average – 1.23 x 2.30 μ.
range – 1.01 - 1.40 x 1.92 - 2.82 μ.

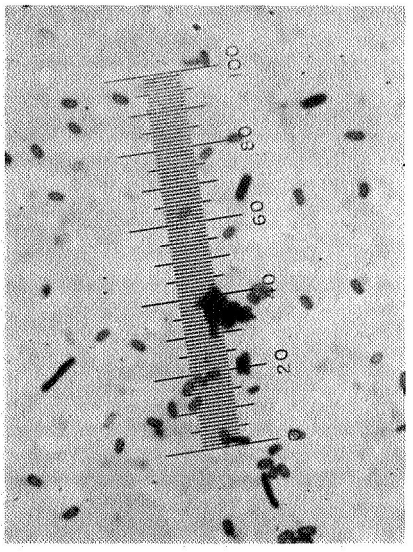
MOTILITY: age 1 DA. NEGATIVE.
Flagella:

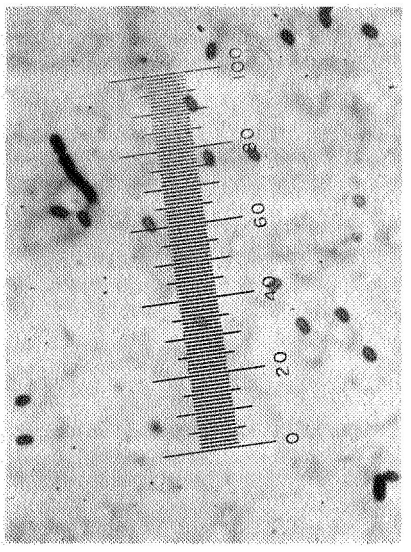
SPORE:



OTHER STAINS:
~~Acid-fast:~~
Capsule:

18; 24; 48 GRAM:





196 AA-1

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 1 DA.

Macroscopic

Size: 3 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | PEARL | 3ba |
| Potato slant | BAMBOO | 2gc |
| – | | |
| – | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 12 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: GOOD.

Fat agar:

Glucose-nitrate agar: GROWTH.

GLUCOSE-NUTRIENT AGAR: EXCELLENT.

NUTRIENT AGAR: GOOD.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: SLIGHT GROWTH. pH = 7.0.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at ~~10~~ 3.5°C. +, 20°C. +, 28°C. +, 37° +, 45°C. +, 55°C. +.

SOLE CARBON SOURCE: age 7 DA.

| | |
|---|---|
| Glucose: *positive, negative.* | 7 DA. |
| Sucrose: *positive, negative.* | 7 DA. |
| Xylose: *positive, negative.* | 7 DA. |
| Citrate: *positive, negative.* | 7 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 7 DA. |
| MANNITOL: POSITIVE. | 7 DA. |
| LACTOSE: POSITIVE. | 7 DA. |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$ +, $NH_4^+$ ___, *gas* ___, *negative.* | 1 DA. |
| Methylene blue: *positive, negative.* | 16 DA. |
| Selenite: *positive, negative.* | ___ |
| Tellurite: *positive, negative.* | ___ |
| – | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Sucrose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Lactose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Xylose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Mannitol: *acid* +, *alkaline, neutral, gas.* | 7 DA. |
| – | |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | 7 DA. |
| Casein: *positive, negative.* | 12 DA. |
| Fat: *positive, negative.* | 7 DA. |
| Starch: *positive, negative.* | 10 DA. |
| Cellulose: *positive, negative.* | ___ |
| Urea: *positive, negative.* | ___ |

TOLERANCES:

| | |
|---|---|
| Salt: 2%—*positive, negative.* | 7 DA. |
| 7%—*positive, negative.* | |
| 10%—*positive, negative.* | |
| pH: *acid* ___, *alkaline* ___. | ___ |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 20 DA. |
| Curd: *acid, alkaline, absent, gas.* | 20 DA. |
| Peptionization: *positive, negative.* | 20 DA. |
| Reduction: *positive, negative.* | 20 DA. |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from ___: *positive, negative.* | ___ |
| $NH_4^+$ from peptone: *positive, negative.* | 7 DA. |
| Acetylmethylcarbinol: *positive, negative.* | 15 DA. |
| Indol: *positive, negative.* | 23 DA. |
| Methyl red: *positive, negative.* | 15 DA. |

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
(JPL–NASA)

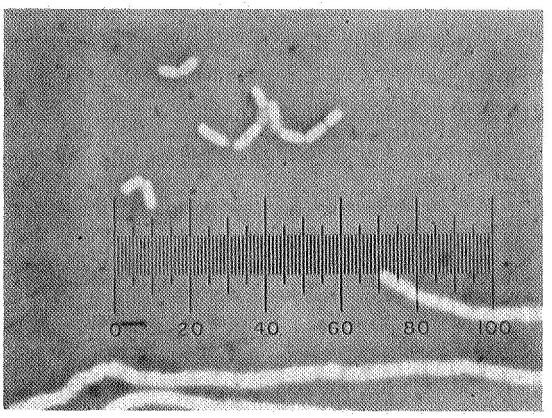
# Descriptive Chart

| 196Ac | Trypticase Soy Agar | Little Lake, California |
|---|---|---|
| (code number) | (medium) | (source) |
| Bacillus megaterium. | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*

MORPHOLOGY:
Form: *rods, ends* ROUND,
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* – 1.32 x 3.19 μ.
*range* – 1.19–1.46 x 2.25–3.81 μ.
Irregular forms:

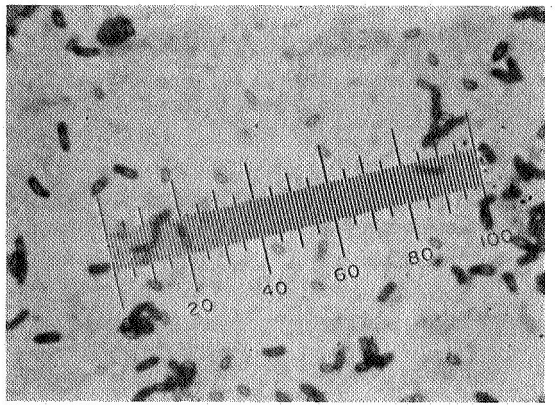
NIGROSIN:




GRAM REACTION:
18 hrs: 100% POSITIVE.
24 hrs: 100 % POSITIVE.
48 hrs: 100 % POSITIVE.

PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average – 1.11 x 2.30 μ.
range – 1.04–1.22 x 1.72–3.19 μ.

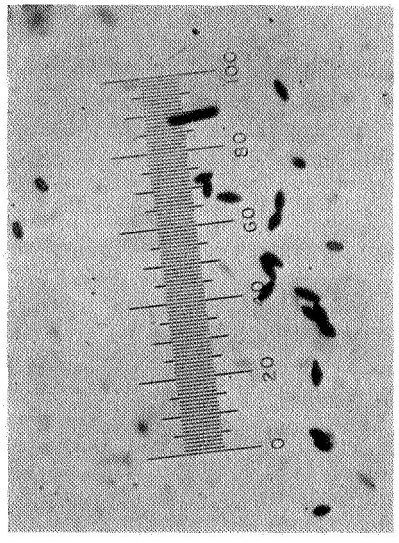
SPORE:

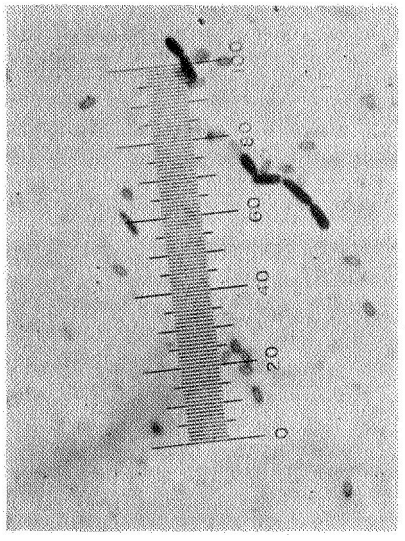


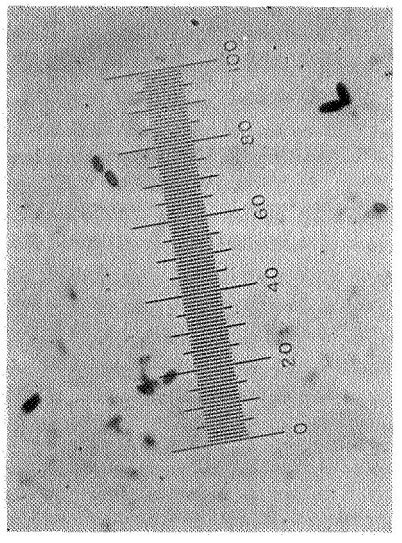

MOTILITY: age 1 DA. NEGATIVE.
Flagella:

OTHER STAINS:
~~Acid fast:~~

18; 24; 48 GRAM:



196 Ac

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 2 DA.

Macroscopic

Size: 3 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | PEARL | 3ba |
| Potato slant | LT. IVORY | 2ca |
| – | | |
| – | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 12 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: GOOD.

Fat agar:

Glucose-nitrate agar: GOOD.

GLUCOSE-NUTRIENT AGAR: EXCELLENT.

NUTRIENT AGAR: GOOD.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: POSITIVE GROWTH. pH 6.8.

–

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at ~~10°C.~~ 5°C. +, 20°C. +, 28°C. +, 37° +, 45°C. +, 55°C. +.

SOLE CARBON SOURCE: age 7 DA.

Glucose: *positive, negative.* 7 DA.

Sucrose: *positive, negative.* 7 DA.

Xylose: *positive, negative.* 7 DA.

Citrate: *positive, negative.* 7 DA.

$NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* _____

MANNITOL: POSITIVE. 7 DA.

LACTOSE: POSITIVE. 7 DA.

REDUCTIONS:

Nitrate: $NO_2^-$ +, $NH_4^+$ ____, *gas* ____, *negative.* 1 DA.

Methylene blue: *positive, negative.* 16 DA.

Selenite: *positive, negative.* _____

Tellurite: *positive, negative.* _____

–

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Sucrose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Lactose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Xylose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Mannitol: *acid* +, *alkaline, neutral, gas.* 7 DA.

–

HYDROLYSIS:

Gelatin: *positive, negative.* 7 DA.

Casein: *positive, negative.* 12 DA.

Fat: *positive, negative.* 7 DA.

Starch: *positive, negative.* 7 DA.

Cellulose: *positive, negative.* _____

Urea: *positive, negative.* _____

TOLERANCES:

Salt: 2%—*positive, negative.* 7 DA.

7%—*positive, negative.*

10%—*positive, negative.*

pH: *acid* _____, *alkaline* _____. _____

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.* 20 DA.

Curd: *acid, alkaline, absent, gas.* 20 DA.

Peptionization: *positive, negative.* 20 DA.

Reduction: *positive, negative.* 20 DA.

OTHER REACTIONS:

$H_2S$ from _____: *positive, negative.* _____

$NH_4^+$ from peptone: *positive, negative.* 7 DA

Acetylmethylcarbinol: *positive, negative.* 15 DA.

Indol: *positive, negative.* 23 DA.

Methyl red: *positive, negative.* 15 DA.

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
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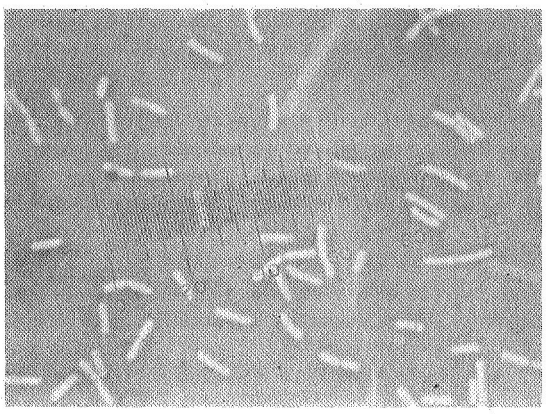
# Descriptive Chart

| | | |
|---|---|---|
| 197Ab (code number) | Trypticase Soy Agar (medium) | Little Lake, California (source) |
| Bacillus megaterium. (name of organism) | 28°C. (temperature) | W.B. Bollen (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*



MORPHOLOGY:

Form: *rods, ends* ROUND,
*filaments, cocci, spirals,*
*branching* _____.

Size: *average* – 0.92 x 3.27 μ.
*range* – 0.72 – 1.04 x 2.56 – 3.32

Irregular forms:

GRAM REACTION: NIGROSIN:

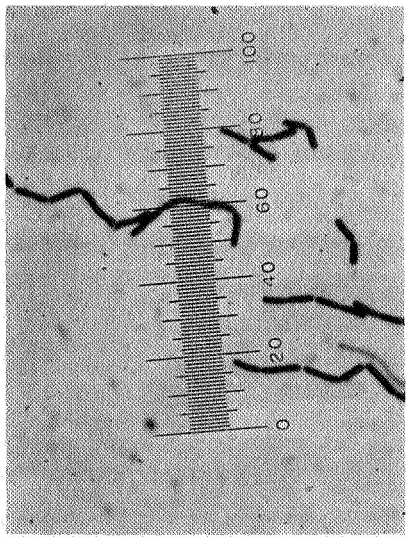
18 hrs: 100 % POSITIVE.
24 hrs: 100 % POSITIVE.
48 hrs: 100 % POSITIVE.

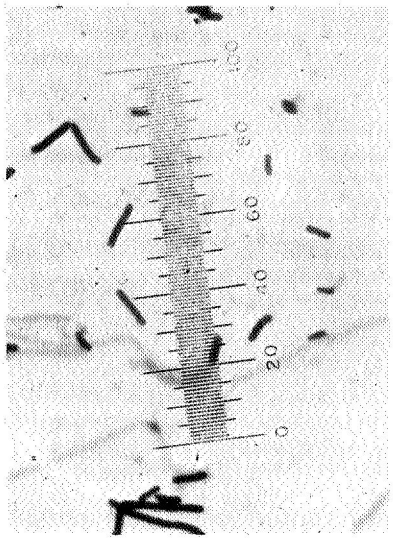
PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.

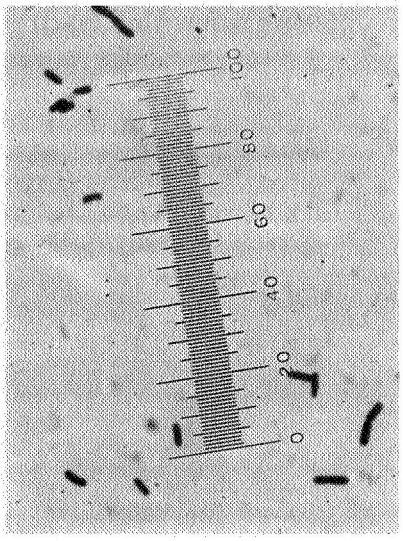
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average –
range –

MOTILITY: age 2 DA. NEGATIVE.
Flagella:

OTHER STAINS: 18, 24, 48 HR. GRAM:
~~Acid fast:~~







197AB

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 1 DA.

Macroscopic

Size: 2 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | CREAM | 1½ CA |
| Potato slant | NO GROWTH. | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 8 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: GOOD.

Fat agar:

Glucose-nitrate agar: FAIR GROWTH.

GLUCOSE-NUTRIENT AGAR: EXCELLENT.

NUTRIENT AGAR: EXCELLENT.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: GROWTH.

DNA

G:C

G+C moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA

Growth at 5.5°C. +, 20°C. +, 28°C. +, 37° +, 43°C. +, 55°C. +.

SOLE CARBON SOURCE: age 7 DA.

| | |
|---|---|
| Glucose: *positive, negative.* | 7 DA. |
| Sucrose: *positive, negative.* | 7 DA. |
| Xylose: *positive, negative.* | 7 DA. |
| Citrate: *positive, negative.* | 2 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 7 DA. |
| MANNITOL: POSITIVE. | 7 DA. |
| LACTOSE: POSITIVE. | 7 DA. |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$, $NH_4^+$, *gas*, *negative.* | 11 DA. |
| Methylene blue: *positive, negative.* | 16 DA. |
| Selenite: *positive, negative.* | |
| Tellurite: *positive, negative.* | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid* +, *alkaline*, *neutral, gas.* | 7 DA. |
| Sucrose: *acid* +, *alkaline*, *neutral, gas.* | 7 DA. |
| Lactose: *acid*, *alkaline* +, *neutral, gas.* | 7 DA. |
| Xylose: *acid*, *alkaline* +, *neutral, gas.* | 7 DA. |
| Mannitol: *acid* +, *alkaline, neutral, gas.* | 7 DA. |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | |
| Casein: *positive, negative.* | 7 DA. |
| Fat: *positive, negative.* | 7 DA. |
| Starch: *positive, negative.* NARROW | 7 DA. |
| Cellulose: *positive, negative.* | |
| Urea: *positive, negative.* | |

TOLERANCES:

| | |
|---|---|
| Salt: 2%—*positive, negative.* | 7 DA. |
| 7%—*positive, negative.* | |
| 10%—*positive, negative.* | |
| pH: *acid*, *alkaline*. | |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 20 DA. |
| Curd: *acid, alkaline, absent, gas.* | 20 DA. |
| Peptionization: *positive, negative.* | 20 DA. |
| Reduction: *positive, negative.* | 4 DA. |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from ____: *positive, negative.* | |
| $NH_4^+$ from peptone: *positive, negative.* | 7 DA. |
| Acetylmethylcarbinol: *positive, negative.* | 17 DA. |
| Indol: *positive, negative.* | 23 DA. |
| Methyl red: *positive, negative.* | 17 DA. |

OREGON STATE UNIVERSITY
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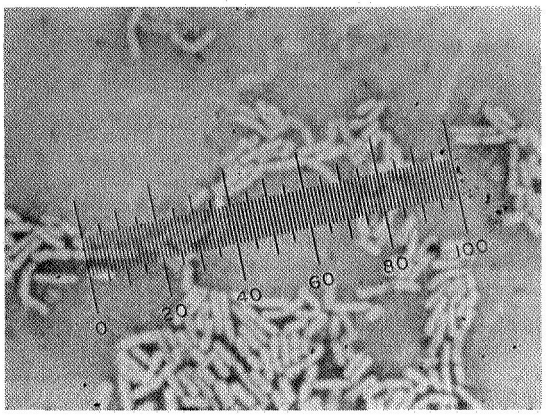
(JPL–NASA)

# Descriptive Chart

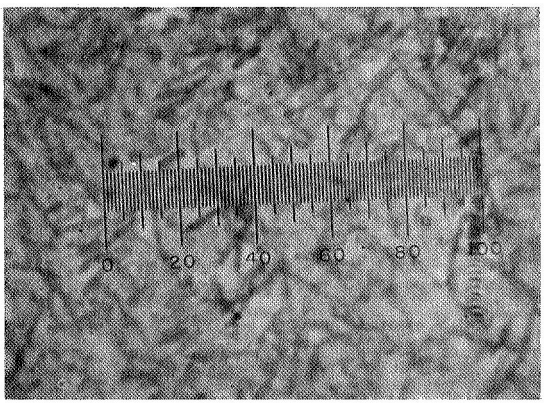
| 197Bc | Trypticase Soy Agar | Little Lake, California. |
|---|---|---|
| (code number) | (medium) | (source) |
| Bacillus megaterium | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*




MORPHOLOGY:
Form: *rods, ends* ROUND,
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* – 1.26 x 3.33μ.
*range* – 1.03-1.65 x 2.71 - 4.07μ.
Irregular forms:

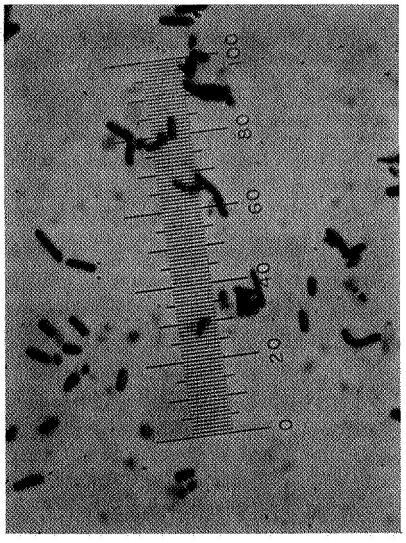
GRAM REACTION: NIGROSIN:
18 hrs: 100% POSITIVE.
24 hrs: 100% POSITIVE.
48 hrs: 100% POSITIVE.

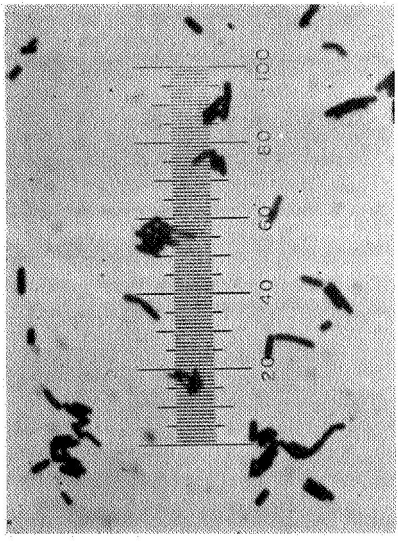



PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average – 0.87 x 1.51μ.
range – 0.68-1.02 x 1.35-1.69μ.

MOTILITY: age 1 DA. NEGATIVE. SPORE:
Flagella:

OTHER STAINS:
~~Acid fast:~~ 18; 24; 48 hr. GRAM:





197 Bc

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 1 DA.

Macroscopic

Size: 2 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | BAMBOO | 2Fb |
| Potato slant | LT. BEIGE | 3ec |
| – | | |
| – | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 8 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: MODERATE.

Fat agar:

Glucose-nitrate agar: SCANT.

GLUCOSE-NUTRIENT AGAR: GOOD.

NUTRIENT AGAR: MODERATE.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: SLIGHT GROWTH. pH 4.2.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at ~~10~~ 5.3°C. –, 20°C. +, 28°C. +, 37° +, 45°C. +, 55°C. +.

SOLE CARBON SOURCE: age 7 DA.

Glucose: *positive, negative.* 7 DA.

Sucrose: *positive, negative.* 7 DA.

Xylose: *positive, negative.* 7 DA.

Citrate: *positive, negative.* 7 DA.

$NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* 7 DA.

MANNITOL: POSITIVE. 7 DA.

LACTOSE: POSITIVE. 7 DA.

REDUCTIONS:

Nitrate: $NO_2^-$ ____, $NH_4^+$ ____, *gas* ____, *negative.* 11 DA.

Methylene blue: *positive, negative.* 3 DA.

Selenite: *positive, negative.* ____

Tellurite: *positive, negative.* ____

–

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Sucrose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Lactose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Xylose: *acid* +, *alkaline* ____, *neutral, gas.* 7 DA.

Mannitol: *acid* +, *alkaline, neutral, gas.* 7 DA.

–

HYDROLYSIS:

Gelatin: *positive, negative.* 20 DA.

Casein: *positive, negative.* 7 DA.

Fat: *positive, negative.* 7 DA.

Starch: *positive, negative.* 7 DA.

Cellulose: *positive, negative.* ____

Urea: *positive, negative.* ____

TOLERANCES:

Salt: 2%—*positive, negative.* 7 DA.

7%—*positive, negative.*

10%—*positive, negative.*

pH: *acid* ____, *alkaline* ____. ____

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.* 9 DA.

Curd: *acid, alkaline, absent, gas.* 30 DA.

Peptionization: *positive, negative.* 12 DA.

Reduction: *positive, negative.* 7 DA.

OTHER REACTIONS:

$H_2S$ from ____: *positive, negative.* ____

$NH_4^+$ from peptone: *positive, negative.* 7 DA.

Acetylmethylcarbinol: *positive, negative.* 15 DA.

Indol: *positive, negative.* 23 DA.

Methyl red: *positive, negative.* 15 DA.

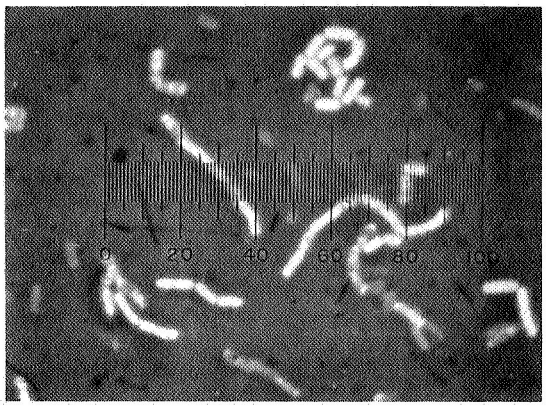
OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
(JPL–NASA)

# Descriptive Chart

| 300Aa | Trypticase Soy Agar | Little Lake, California. |
|---|---|---|
| (code number) | (medium) | (source) |
| Bacillus megaterium. | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*

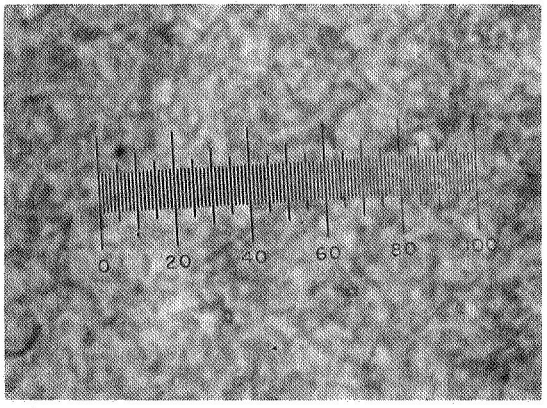
MORPHOLOGY:
Form: *rods, ends* ROUND,
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* – 1.18 x 3.21μ.
*range* – 1.02-1.20 x 2.62 - 3.76μ.
Irregular forms:




NIGROSIN:

GRAM REACTION:
18 hrs: 100 % POSITIVE.
24 hrs: 100 % POSITIVE.
48 hrs: 100 % POSITIVE.

PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen,.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average – 0.77 x 1.22μ.
range – 0.62 - 0.99 x 1.01 - 1.76μ.

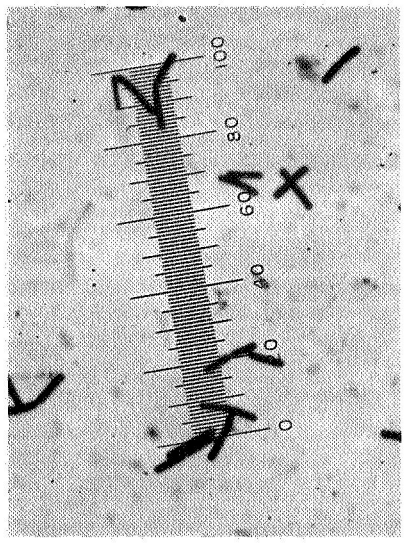


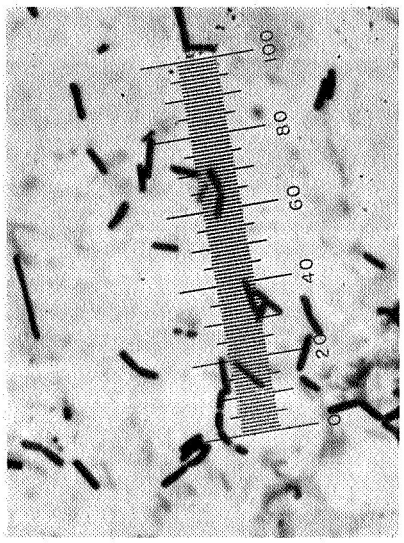

MOTILITY: age 1 DA. NEGATIVE.
Flagella:

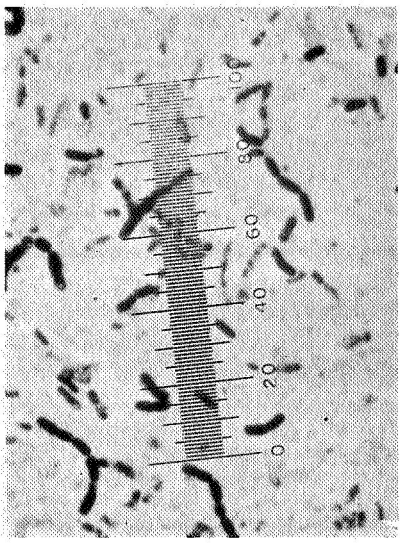
SPORE:

OTHER STAINS:
~~Acid fast:~~

18; 24; 48 hr. GRAM:



30DAA

## II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 3 DA.

Macroscopic

Size: 4 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | CREAM | 1½ ca |
| Potato slant | PEARL PINK | 3 ca |
| — | | |
| — | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling $H_2S$.

GELATIN STAB: age 4 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: EXCELLENT.

Fat agar:

Glucose-nitrate agar: FAIR.

GLUCOSE-NUTRIENT AGAR: EXCELLENT.

NUTRIENT AGAR: GOOD.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: SLIGHT GROWTH. pH 7.0.

DNA

G:C _____

G+C _____ moles %

## III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at 5°C. −, 20°C. +, 28°C. +, 37° +, 45°C. +, 55°C. ±.

SOLE CARBON SOURCE: age 7 DA.

| | |
|---|---|
| Glucose: *positive, negative.* | 7 DA. |
| Sucrose: *positive, negative.* | 7 DA. |
| Xylose: *positive, negative.* | 7 DA. |
| Citrate: *positive, negative.* | 7 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 7 DA. |
| MANNITOL: POSITIVE. | 7 DA. |
| LACTOSE: POSITIVE. | 7 DA. |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$ ____, $NH_4^+$ ____, *gas* ____, *negative.* | 11 DA. |
| Methylene blue: *positive, negative.* | 6 DA. |
| Selenite: *positive, negative.* | ____ |
| Tellurite: *positive, negative.* | ____ |
| — | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid* +, *alkaline* ____, *neutral, gas.* | 7 DA. |
| Sucrose: *acid* +, *alkaline* ____, *neutral, gas.* | 7 DA. |
| Lactose: *acid* ____, *alkaline* +, *neutral, gas.* | 30 DA. |
| Xylose: *acid* ____, *alkaline* ____, *neutral, gas.* | 30 DA. |
| Mannitol: *acid* ____, *alkaline, neutral, gas.* | 30 DA. |
| — | |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | 7 DA. |
| Casein: *positive, negative.* | 12 DA. |
| Fat: *positive, negative.* | 7 DA. |
| Starch: *positive, negative.* | 7 DA. |
| Cellulose: *positive, negative.* | ____ |
| Urea: *positive, negative.* | ____ |

TOLERANCES:

| | |
|---|---|
| Salt: 2%—*positive, negative.* | 7 DA. |
| 7%—*positive, negative.* | |
| 10%—*positive, negative.* | |
| pH: *acid* ____, *alkaline* ____ | ____ |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 10 DA. |
| Curd: *acid, alkaline, absent, gas.* | 30 DA. |
| Peptionization: *positive, negative.* | 10 DA. |
| Reduction: *positive, negative.* | 7 DA. |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from ____: *positive, negative.* | ____ |
| $NH_4^+$ from peptone: *positive, negative.* | 7 DA. |
| Acetylmethylcarbinol: *positive, negative.* | 15 DA. |
| Indol: *positive, negative.* | 23 DA. |
| Methyl red: *positive, negative.* | 15 DA. |

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
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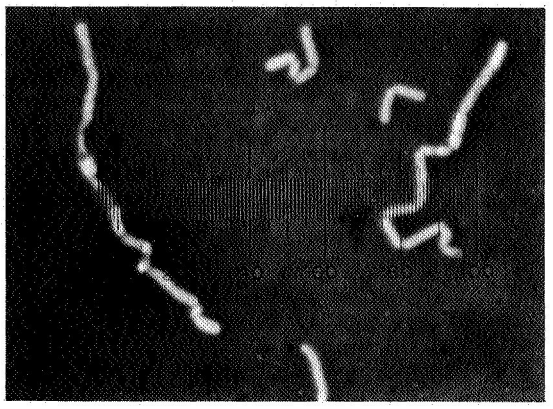
# Descriptive Chart

| 300Ae | Trypticase Soy Agar | Little Lake, California. |
|---|---|---|
| (code number) | (medium) | (source) |
| Bacillus megaterium | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

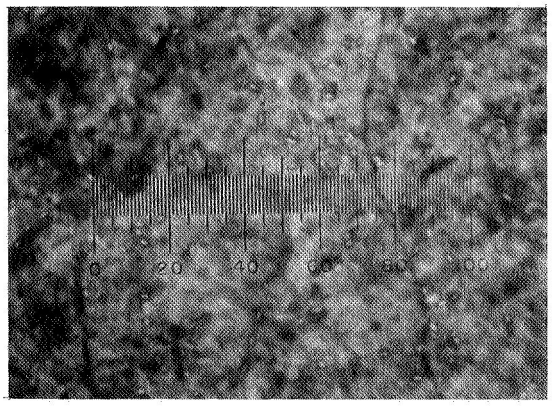
## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*



MORPHOLOGY:
Form: *rods, ends* ROUND,
*filaments, cocci, spirals,*
*branching* _____.
Size: *average* – 1.21 x 2.42μ.
*range* – 0.91 - 1.38 x 1.76 - 3.03μ.
Irregular forms:

NIGROSIN:

GRAM REACTION:
18 hrs: 100% POSITIVE.
24 hrs: 100% POSITIVE.
48 hrs: 100% POSITIVE.



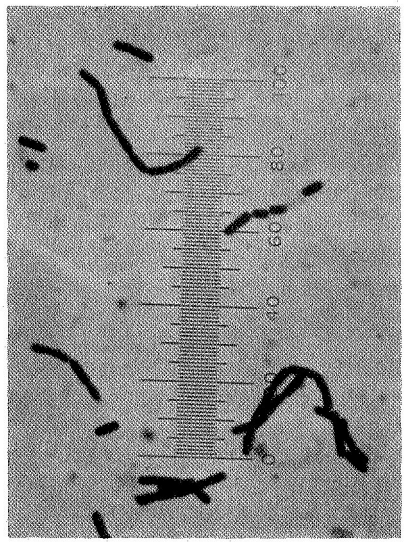
PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.
Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: average – 0.79 x 1.35μ.
range – 0.70 - 0.88 x 1.26 - 1.70μ.

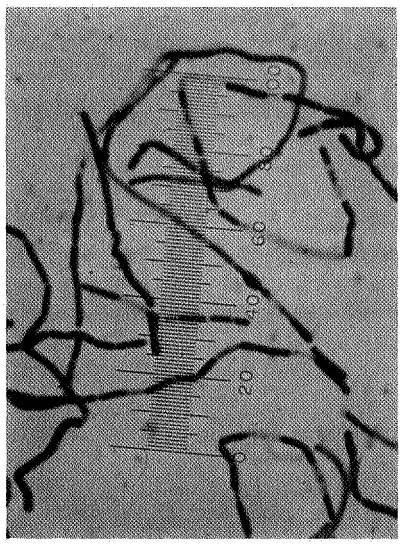
SPORE:

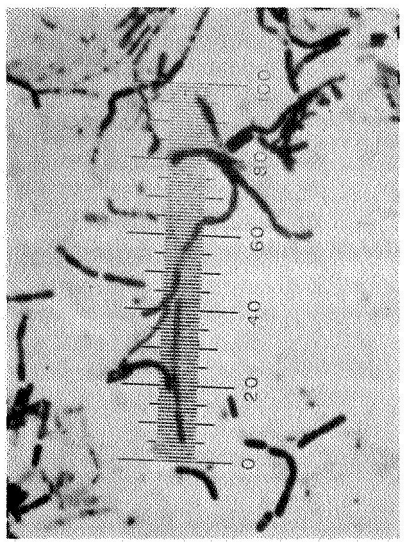
MOTILITY: age 1 DA: NEGATIVE.
Flagella: 4 DA: NEGATIVE.

OTHER STAINS:
~~Acid-fast~~:

18; 24; 48 hr. GRAM:







300AE

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 5 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 2 DA.

Macroscopic

Size: 3 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | PEARL PINK | 3CA |
| Potato slant | | |
| – | | |
| – | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 6 DA.

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

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: GOOD.

Fat agar:

Glucose-nitrate agar: SCANT.

GLUCOSE-NUTRIENT AGAR: SCANT.

NUTRIENT AGAR: FAINT.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: GROWTH.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at ~~10~~ 5.5°C. –, 20°C. +, 28°C. +, 35° +, 45°C. –, 55°C. –.

SOLE CARBON SOURCE: age _____.

| | |
|---|---|
| Glucose: *positive, negative.* | 7 DA. |
| Sucrose: *positive, negative.* | 7 DA. |
| Xylose: *positive, negative.* | 7 DA. |
| Citrate: *positive, negative.* | 2 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 5 DA. |
| MANNITOL: POSITIVE. | 7 DA. |
| LACTOSE: POSITIVE. | 7 DA. |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$ ____, $NH_4^+$ ____, *gas* ____, *negative.* | 15 DA. |
| Methylene blue: *positive, negative.* | 9 DA. |
| Selenite: *positive, negative.* | |
| Tellurite: *positive, negative.* | |
| – | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Sucrose: *acid* +, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Lactose: *acid* ___, *alkaline* +, *neutral, gas.* | 7 DA. |
| Xylose: *acid* ___, *alkaline* ___, *neutral, gas.* | 7 DA. |
| Mannitol: *acid* +, *alkaline, neutral, gas.* | 7 DA. |
| – | |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | 7 DA. |
| Casein: *positive, negative.* | 2 DA. |
| Fat: *positive, negative.* | 14 DA. |
| Starch: *positive, negative.* | 14 DA. |
| Cellulose: *positive, negative.* | |
| Urea: *positive, negative.* | |

TOLERANCES:

| | |
|---|---|
| Salt: 2%–*positive, negative.* | 5 DA. |
| 7%–*positive, negative.* | |
| 10%–*positive, negative.* | |
| pH: *acid* _____, *alkaline* _____. | |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 20 DA. |
| Curd: *acid, alkaline, absent, gas.* | 20 DA. |
| Peptionization: *positive, negative.* | 20 DA. |
| Reduction: *positive, negative.* | 20 DA. |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from _____: *positive, negative.* | |
| $NH_4^+$ from peptone: *positive, negative.* | 6 DA. |
| Acetylmethylcarbinol: *positive, negative.* | 17 DA. |
| Indol: *positive, negative.* | 23 DA. |
| Methyl red: *positive, negative.* | 17 DA. |

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
(JPL–NASA)

# Descriptive Chart

| 304Aa | Trypticase Soy Agar | Little Lake, California. |
|---|---|---|
| (code number) | (medium) | (source) |
| Bacillus subtilis var. niger | 28°C. | W.B. Bollen |
| (name of organism) | (temperature) | (studied by) |

## I. *STAINING & MORPHOLOGICAL CHARACTERISTICS*

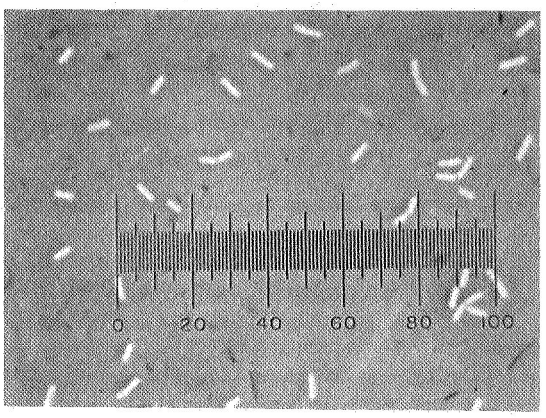
MORPHOLOGY:

Form: *rods, ends* ROUND, *filaments, cocci, spirals, branching* _____.

Size: *average* – 0.75 x 2.65 μ.
*range* – 0.62-0.82 x 2.19-3.18 μ.

Irregular forms:

NIGROSIN:




GRAM REACTION:

18 hrs: 100% POSITIVE.
24 hrs: 100% POSITIVE.
48 hrs: 100% POSITIVE.

PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.

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

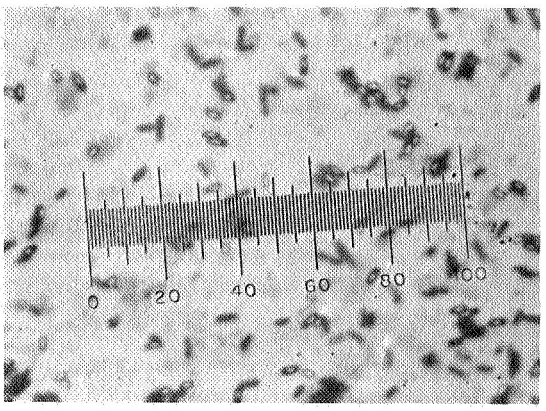
Endospores: *swollen, not swollen.*

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

Shape: *spherical, ellipsoidal, cylindrical, oval.*

size: average – 0.99 x 1.94μ.
range – 0.92-1.18 x 1.42 - 2.55μ.

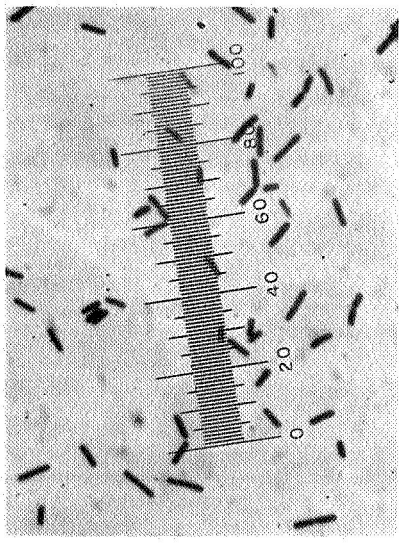
SPORE:

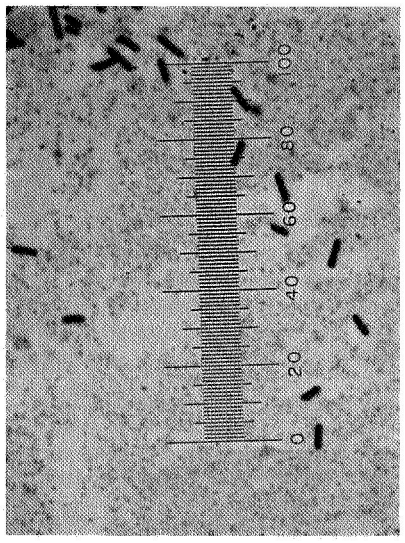


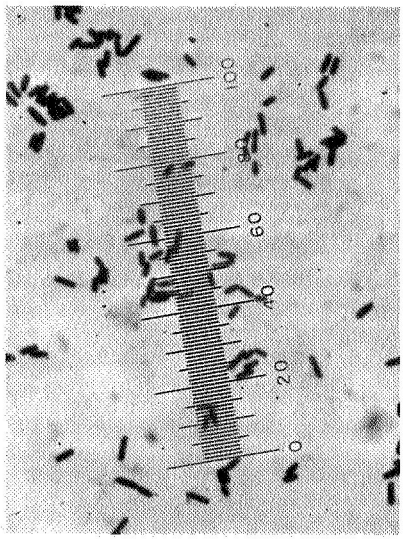

MOTILITY: age 1 DAY NEGATIVE.
Flagella:

OTHER STAINS:
~~Acid fast~~

18; 24; 48 hr. GRAM:



304 Aa

## II. *CULTURAL CHARACTERISTICS*

AGAR STROKE: age 3 DA.

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

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

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 1 DA.

Macroscopic

Size: 2 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

| (medium) | (color) | (CHM No.) |
|---|---|---|
| Trypticase soy agar | PEARL | 3ba |
| Potato slant | BLACK PLUM | 10 po |
| — | | |
| — | | |

NUTRIENT BROTH: age 4 DA.

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

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

Subsurface growth: *none, granular, turbid.*

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

Odor: resembling NONE.

GELATIN STAB: age 3 DA.

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

Rate: *fast, moderate, low.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: EXCELLENT.

Fat agar:

Glucose-nitrate agar: MODERATE.

GLUCOSE-NUTRIENT AGAR: EXCELLENT.

NUTRIENT AGAR: EXCELLENT.

NUTRIENT AGAR + TYROSINE: BLACK GROWTH.

ANAEROBIC NITRATE BROTH: NO GAS.

ANAEROBIC GLUCOSE BROTH: GROWTH. pH 6.8.

DNA

G:C _____

G+C _____ moles %

## III. *PHYSIOLOGICAL CHARACTERISTICS*

RELATIONSHIP TO $O_2$: *aerobic, anaerobic, facultative, microaerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 3 DA.

Growth at 15°C. +, 20°C. +, 28°C. +, 37° +, 45°C. +, 55°C. ±.

SOLE CARBON SOURCE: age _____.

| | |
|---|---|
| Glucose: *positive, negative.* | 7 DA. |
| Sucrose: *positive, negative.* | 7 DA. |
| Xylose: *positive, negative.* | 7 DA. |
| Citrate: *positive, negative.* | 7 DA. |
| $NH_4^+$ AS SOLE NITROGEN SOURCE: *positive, negative.* | 7 DA. |
| MANNITOL: POSITIVE. | 7 DA. |
| LACTOSE: POSITIVE. | 7 DA. |

REDUCTIONS:

| | |
|---|---|
| Nitrate: $NO_2^-$ +, $NH_4^+$ ____, *gas* ____, *negative.* | 1 DA. |
| Methylene blue: *positive, negative.* | 1 DA. |
| Selenite: *positive, negative.* | ____ |
| Tellurite: *positive, negative.* | ____ |
| — | |

OXIDATIVE-FERMENTATIVE REACTIONS

| | |
|---|---|
| Glucose: *acid* +, *alkaline* ____, *neutral, gas.* | 7 DA. |
| Sucrose: *acid* +, *alkaline* ____, *neutral, gas.* | 7 DA. |
| Lactose: *acid* ____, *alkaline* +, *neutral, gas.* | 7 DA. |
| Xylose: *acid* ____, *alkaline* +, *neutral, gas.* | 7 DA. |
| Mannitol: *acid* +, *alkaline, neutral, gas.* | 7 DA. |
| — | |

HYDROLYSIS:

| | |
|---|---|
| Gelatin: *positive, negative.* | 7 DA. |
| Casein: *positive, negative.* | 7 DA. |
| Fat: *positive, negative.* | 7 DA. |
| Starch: *positive, negative.* | 7 DA. |
| Cellulose: *positive, negative.* | ____ |
| Urea: *positive, negative.* | ____ |

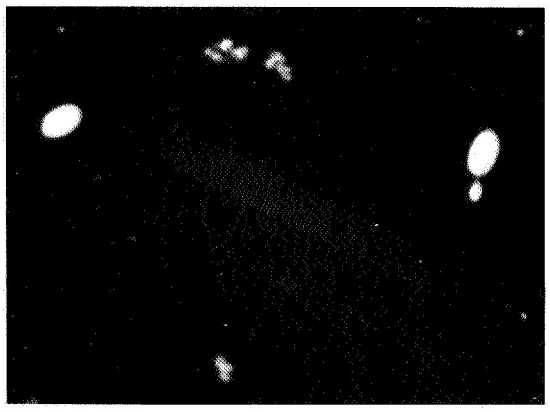
TOLERANCES:

| | |
|---|---|
| Salt: 2%—*positive, negative.* | 7 DA. |
| 7%—*positive, negative.* | |
| 10%—*positive, negative.* | |
| pH: *acid* ____, *alkaline* ____. | ____ |

LITMUS MILK REACTIONS:

| | |
|---|---|
| Reaction: *acid, alkaline, neutral.* | 3 DA. |
| Curd: *acid, alkaline, absent, gas.* | 30 DA. |
| Peptionization: *positive, negative.* | 2 DA. |
| Reduction: *positive, negative.* | 3 DA. |

OTHER REACTIONS:

| | |
|---|---|
| $H_2S$ from ____: *positive, negative.* | ____ |
| $NH_4^+$ from peptone: *positive, negative.* | 7 DA. |
| Acetylmethylcarbinol: *positive, negative.* | 15 DA. |
| Indol: *positive, negative.* | 23 DA. |
| Methyl red: *positive, negative.* | 15 DA. |

Nigrosin stain of 24 hour culture of isolant, 303Bb. Isolant, 300Bd is the same.