PROGRESS REPORT

Microorganism Study
JPL Contract No. 950783, under NAS 7-100
Prof. W.B. Bollen, Microbiologist
Oregon State University
Corvallis, Oregon
August 20, 1965
Descriptive charts for 12 more isolants from the first group of 31 cultures sent to us are presented herewith. All 12 are soil diphtheroids; the only available genus for these is *Corynebacterium*, into which they fall readily with respect to morphology and general physiology. However, they differ from all described species, many of the biochemical reactions and cultural characters, especially pigmentation, being different. All species described in Bergey's Manual are animal pathogens or parasites, or plant pathogens. Soil diphtheroids are mentioned in the literature but none have been systematically described and named. They undoubtedly constitute a part of Winogradsky's unclassified autochthonous microflora and a new genus should be established for them. Most of the second group of cultures you submitted are also soil diphtheroids. While most of the major tests on these have been completed, photomicrographs and measurements remain to be done. When these descriptions, and descriptions of other soil diphtheroids that may be found in subsequent cultures sent to us, are completed, a complete key will be prepared and an appropriate new genus and species names will be suggested.

From the Key presented herewith, and preceding the descriptive charts, it is apparent that the main differentiating characters are size, hydrolysis of gelatin, and reduction of nitrates. The subsequent keying characters are less significant and may be variable. It seems doubtful if the 12 isolants represent even six justifiable species. Cultures 15B2 and 15B3 differ only in color, and but slightly; on different media the colors would probably be different than on trypticase soy agar and would perhaps be even more alike, suggesting variants of a single species.

Seventeen of the third group of 48 isolants have failed to grow on transfer to TSA or in enrichment media. Of the 17, most are small cocci or short rods; five show branching.

The fourth group of isolants, from Hilgard's soils, are all growing except No. 82A. Cultural characters only have been completed.

Culture work is just starting on the recently received fifth group of isolants from the African and Chilean desert soils.
Corynebacterium. Aerobic. Gram-positive. non-
spore-forming. Non-motile. Usually straight; but
occasionally slightly curved, club-shaped, and ir-
regularly stained segments. Angular and palisade
formations of cells may follow characteristic snap-
ning division. Catalase-positive. Utilize NH₄ as
sole nitrogen source. Do not hydrolyze fat, nor
produce indol, acetyl methyl carbinol, or hydrogen
sulfide. May or may not liquefy gelatin, reduce
nitrates to nitrites, or ferment sugars. If sugars
fermented, only slight amount of acid produced.
I. Large size. 2.5-3.6 x 0.8-0.9 μ.  
Casein, gelatin, and starch not hydrolyzed. Urea hydrolyzed.  
Ammonia from peptone.  

A. Nitrates reduced. Methylene blue reduced.  
1. Salt tolerance 2%...................... 15A1  
2. Salt tolerance 10%.  
   a. Dull................................... 19X  
   b. Glistening.  
   (1). Color Lt. Mellon Yellow... 15A  
   (2). Color Lt. Apricot............. 19G1  

B. Nitrates not reduced.  
1. Methylene blue reduced.  
   a. Gelatin liquefaction............. 122B  
   b. No gelatin liquefaction......... 19Y  
2. Methylene blue not reduced........... 19E1  

II. Small size. 0.9-1.1 x 0.4-0.6 μ.  

A. Gelatin hydrolyzed..................... 16  
B. Gelatin not hydrolyzed.  
1. Nitrate reduced. Starch positive... 13C  
   a. Ammonia from peptone............. 14D  
   b. No ammonia from peptone.  
   (1). Color Flame...................... 15B3  
   (2). Color Cherry...................... 15B2
III. Systematic study of isolants 14E, 19E2, and 20F has not been completed because the cultures have failed to continue to grow on transfer. Sugar utilization, colony descriptions, photography, and measurements remain unfinished. Characters so far determined indicate these isolants are soil diphtheroids; 14E falls into the small size group, 19E2 and 20F are in the large group.
Name of organism: **Corynebacterium sp.**

Source: White Mountain

**CELL MORPHOLOGY**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Form</th>
<th>Arrangement</th>
<th>Motility in broth</th>
<th>Sporulation</th>
<th>Age</th>
<th>Temp.</th>
<th>Sketches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypticase soy agar</td>
<td>Strepococci, diplococci, micrococci, sarcinae, rods, cocci, spores, branched rods, filaments</td>
<td>No flagella</td>
<td>24 hr.</td>
<td>Age: 24 hr.</td>
<td>28°C</td>
<td>SEE PAGE 3 FOR PHOTOMICROGRAPHS</td>
<td></td>
</tr>
</tbody>
</table>

**STAINING CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Gram</th>
<th>Age</th>
<th>Special stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>24 hr. Method: Kopeloff's modified</td>
<td></td>
</tr>
</tbody>
</table>

**AGAR STROKE**

<table>
<thead>
<tr>
<th>Age</th>
<th>Temp.</th>
<th>Sketches</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr.</td>
<td>28°C</td>
<td>moderate to abundant after 48 hr.</td>
</tr>
</tbody>
</table>

**AGAR COLONIES**

<table>
<thead>
<tr>
<th>Age</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 days</td>
<td>28°C</td>
</tr>
</tbody>
</table>

**NUTRIENT BROTH**

<table>
<thead>
<tr>
<th>Age</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 days</td>
<td>28°C</td>
</tr>
</tbody>
</table>

**GELATIN STAB**

<table>
<thead>
<tr>
<th>Age</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 days</td>
<td>25°C</td>
</tr>
</tbody>
</table>

**OTHER MEDIA**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temp.</th>
<th>Sketches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato slant</td>
<td>61a</td>
<td>Brite Coral Red</td>
</tr>
<tr>
<td>Soybean Infusion agar</td>
<td>4ea</td>
<td>Light Apricot abundant</td>
</tr>
<tr>
<td>Fat agar</td>
<td>4ea</td>
<td>Light Apricot abundant</td>
</tr>
<tr>
<td>Glucose nitrate</td>
<td>4ca</td>
<td>Flesh Pink abundant</td>
</tr>
</tbody>
</table>

**FERMENTATION**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Nutrient Broth</th>
<th>Carbohydrate:</th>
<th>Indicator:</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Lactose</td>
<td>Sucrose</td>
<td>CO₂</td>
<td></td>
</tr>
<tr>
<td>Acid in</td>
<td>2 days</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Acid in</td>
<td>days</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Gas in</td>
<td>2 days</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Gas in</td>
<td>days</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

**ACTION ON MILK**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Temp. 28 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus</td>
<td>35</td>
</tr>
<tr>
<td>Reaction</td>
<td>=</td>
</tr>
<tr>
<td>Acid curd</td>
<td>=</td>
</tr>
<tr>
<td>Rennet curd</td>
<td>=</td>
</tr>
<tr>
<td>Peptonization</td>
<td>=</td>
</tr>
<tr>
<td>Reduction (before coagulation)</td>
<td>=</td>
</tr>
</tbody>
</table>

* Culturally similar to *C. equi*, an animal pathogen.*
ACTION ON NITRATES

Medium: 1% KNO₃ broth  Temp. 28°C.
Nitrite: + d. ; + d. ; + d. 
Gas (N₂): + d. ; + d. ; + d. 

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio iron  Age: 10 day
H₂S: present, absent.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C): present, absent. 
Growth at room temperature (25°C): present, absent. 
Growth at 37°C: present, absent. 
Growth at 50°C: present, absent. 

Pseudomonas: 
Pasteurization survival, 80°C. 10 minutes: Negative

ADDITIONAL TESTS

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

NH₄ from Peptone: Positive
Metabolism: Nonoxidizing-nonfermenter

Acetyl methyl carbinol: Negative

NH₄ as sole Nitrogen source: Positive

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

Methylene blue reduction: Positive

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

Cellulose Digestion: Negative

Selenite-nutrient agar: Positive - slight

INDOLE PRODUCTION

Medium: Selenite broth
Method: Kovac's
Indole: present, absent.

RELATION TO FREE OXYGEN

Catalase - Positive

Medium: TSA & Dextrose
Method: Shake tubes
Age: 10 day

Aerobic growth: absent, present, better than anaerobic growth, poorer than anaerobic growth.
An aerobic growth: present, absent.

Metabolism: Nonoxidizer-nonfermenter
PHOTOMICROGRAPHS

1000x

NTGYSN - 24 hour

GRAM - 18 hour

GRAM - 24 hour

GRAM - 48 hour
Cultural Characteristics of Bacterial Colonies

Culture No. 13C

I. Surface Colonies. Age 8 days, on ASB medium.

a. Microscopic appearance.

1. Size 3 mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.
   Elevation- effuse, flat, raised, convex, rugose.
   Papillate, umbonate, pulvinate.
   Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.
   Habit- compact, spreading.

3. Optical properties:
   (a) Color: Color Harmony Manual No. 550 (Peach)
   (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, filamentous, curled.

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

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

d. Odor. (Specify)
**Name of organism**: Corynebacterium sp.*

**Source**: White Mountain

**Habitat**: Soil

**Date**: August 2, 1965

### Cell Morphology
- **Medium**: Trypticase soy agar, temp. 28°C.
- **Vegetative cells**: Rods, short, straight, curved, or S-shaped.
- **Motility**: No flagella.
- **Spore**: No spores observed.
- **Shape**: Spherical, oval, or irregular.
- **Position**: Central, terminal, or subterminal.

### Staining Characteristics
- **Gram stain**: +
- **Method**: Koeploff's (modified).

### Agar Stroke
- **Temp.**: 28°C.
- **Age**: 24 hr.
- **Agar stroke**: Brite Coral Red.
- **Non-water soluble pigment**: Present.

### Agar Colonies
- **Form**: Smooth, rounded, translucent, rhizoid, irregular.
- **Surface**: Smooth, convex, raised, translucent, opaque.
- **Texture**: Dry, powdery, raised, translucent, opaque.
- **Density**: Sparse, translucent.

### Nutrient Broth
- **Temp.**: 25°C.
- **Age**: 12 days.
- **Surface growth**: None, ring, pellicle, fusculent, membranous.
- **Subsurface growth**: None, turbid, granular.
- **Amount of growth**: None, moderate, abundant.
- **Sediment**: None, granular, fusculent, sticky.

### Gelatin Slab
- **Temp.**: 25°C.
- **Age**: 12 days.

### Other Media
- **Potato slant**: No growth.
- **Soybean Inf. agar**: Moderate growth.
- **Glucose nitrate agar**: Abundant growth.
- **Fat agar**: Abundant growth.

### Fermentation
<table>
<thead>
<tr>
<th>Medium</th>
<th>Temp. 25°C</th>
<th>Lactic</th>
<th>Sucrose</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient broth</td>
<td>1% Carbohydrate: BCP</td>
<td>Glucose</td>
<td>Lactic</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Acid in 10 days</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Acid in days</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Gas in 10 days</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Gas in days</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

### Action on Milk
<table>
<thead>
<tr>
<th>Indicator</th>
<th>Temp. 25°C</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Reaction</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Acid curd</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Rennet curd</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Peptonization</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Reduction (before coagulation)</td>
<td>=</td>
<td></td>
</tr>
</tbody>
</table>

*No agreement with any described species in Bergey's Manual.*
ACTION ON NITRATES
Medium: 1% KNO₃ broth  Temp. 28°C.
Nitrates: ........... d.  ; ........... d.  ; =3......d.
Gas (N): .......... d.  ; .......... d.  ; =3.....d.

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Thio Iron  Temp. 28°C.
H₂S: present, absent.

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

ACTION ON NITRATES
Gas (N): .......... d.  ; .......... d.  ; =3.....d.

INDOLE PRODUCTION
Medium: Tryptophane broth  Age: 10 day  Temp. 28°C.
Method: KOVAC'S
Indole: present, absent.

RELATION TO FREE OXYGEN
Catalase: Positive
Medium: Dextrose-Nutrient Agar  10 days  Temp. 25°C.
Method: Shake tubes

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

PASTEURIZATION survival, 80°C. 10 minutes: Negative

additional tests

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

NH₄ from Peptone:
Metabolism:

Acetyl methyl carbinol:

NH₄ as sole Nitrogen source:
Sole Carbon sources:  Citrate -
                      Glucose -
                      Sucrose -
                      Xylose -

Methylen blue reduction:
Salt tolerances:  2% -
                7% -
                10% -

Cellulose Digestion:
Selenite-nutrient agar:
Cultural Characteristics of Bacterial Colonies

I. Surface Colonies. Age 8 days, on TSA medium.
   a. Microscopic appearance.

   1. Size, 2mm

   2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.
      Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
      Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.
      Habit- compact, spreading.

   3. Optical properties:
      (a) Color: Color Harmony Manual No. 660 \( \text{Brill \ Coral Red} \)
      (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, filamentous, curled.
      2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

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

   d. Odor, cooking cauliflower
Name of organism: Corynebacterium sp.  

**Source:** White Mountain 
**Habitat:** Soil 
**Date:** August 2, 1965

**Descriptions (Underline required terms.)**

**CELL MORPHOLOGY**  
Medium: Trypticase Soy Agar  
Temp: 25 °C.

**Vegetative cells:**
- Form and arrangement: streptococci, diplococci, micrococci, cocci, spirals, branching rods, filaments.
- Mobility: immobile.
- Size: variable.  
- Shape: rectangular forms.
- Sporangia: none, rods, spindles, divided, ill-defined, dense, clump-like.
- Age: 
- Redesporation: spherical, ellipsoid, cylindrical.
- Position: central to eccentric, terminal, subterminal.

**STAINING CHARACTERISTICS**
- Gram: +  
- Age: 24 hr  
- Method: Köpfliff (modified)

**AGAR STROKE**  
Age: 24 hr.  
Temp: 25 °C.
- Amount of growth: scanty, moderate, abundant.
- Form: ellipsoid, ovoid, long, short, rod-like.
- Consistency: viscous, membranous, brittle.
- Chromogenesis: fluorescent, iridescent, photogenic.

**AGAR COLONIES**  
Age: 8 day.  
Temp: 25 °C.
- Form: punctiform, circular, filamentous, rhizoid, irregular.
- Elevation: effuse, flat, raised, convex.
- Surface: smooth, convoluted, rough, domed, regular.
- Margin: entire, undulate, cross, filamentous, curved.
- 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, bullate, mixed, fatty.

**GELATIN STAB**  
Age: 5 day.  
Temp: 25 °C.
- Liquefaction: none, turbid, turbid-like, confluent, napaform, saccate, striiform.
- Rate: slow, moderate, rapid.

**OTHER MEDIA**
- Potato slant: 4ge
- Soybean Infusion agar: White
- Glucose nitrate agar: Pearl Pink
- Fat agar: Clear to White

**FERMENTATION**  
Temp: 25 °C.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Nutrient Broth 1%</th>
<th>Carbohydrate</th>
<th>Indicator: Litmus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Lactose</td>
<td></td>
</tr>
<tr>
<td>Acid in</td>
<td>2 days</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acid in</td>
<td>10 days</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gas in</td>
<td>2 days</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gas in</td>
<td>10 days</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**ACTION ON MILK**  
Temp: 25 °C.

<table>
<thead>
<tr>
<th>Indicator:</th>
<th>Days</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus</td>
<td>±</td>
<td>ALKALINE</td>
</tr>
<tr>
<td>Acid curd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rennet curd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptonization</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Soil G1 theroid; no described species.  
Resembles closely 15A1.
### Action on Nitrates

<table>
<thead>
<tr>
<th>Medium</th>
<th>18KNO₃ broth</th>
<th>Temp. 28°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite:</td>
<td>⬤ d</td>
<td>⬤ 3 d</td>
</tr>
<tr>
<td>Gas (N):</td>
<td>⬤ d</td>
<td>⬤ 3 d</td>
</tr>
</tbody>
</table>

### Indole Production

<table>
<thead>
<tr>
<th>Medium</th>
<th>Tryptophane broth</th>
<th>Age: 10 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>Kovac's</td>
<td>Temp. 28°C</td>
</tr>
<tr>
<td>Indole:</td>
<td>present, absent</td>
<td></td>
</tr>
</tbody>
</table>

### Hydrogen Sulfide Production

<table>
<thead>
<tr>
<th>Medium:</th>
<th>Pb Acetate &amp; Thio-iron</th>
<th>Age: 10 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S:</td>
<td>present, absent</td>
<td></td>
</tr>
<tr>
<td>Temp.</td>
<td>25°C</td>
<td></td>
</tr>
</tbody>
</table>

### Temperature Relations

| Growth in refrigerator (10°C): | present, absent |
| Growth at room temperature (20°C): | present, absent |
| Growth at 37°C: | present, absent |

### Pasteurization Survival

- **Temperature**: 80°C, 10 minutes
- **Result**: Negative

### Additional Tests

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

### NH₄ from Peptone

<table>
<thead>
<tr>
<th>Metabolism:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl methyl carbinol:</td>
</tr>
<tr>
<td>NH₄ as sole Nitrogen source:</td>
</tr>
<tr>
<td>Sole Carbon sources:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

### Methylene blue reduction:

- **Result**: Positive

### Salt tolerances:

- **2%**, **7%**, **10%**

### Cellulose Digestion:

<table>
<thead>
<tr>
<th>Selenite-nutrient agar:</th>
</tr>
</thead>
</table>
Cultural Characteristics of Bacterial Colonies

Culture No. 15A

I. Surface Colonies. Age 8 days, on TSA medium.
   a. Microscopic appearance.

   1. Size, 4mm
   2. Shape: Outline - punctiform, circular, oval, irregular, filamentous, rhizoid.

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

   Topography - smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.

   Habit - compact, spreading.

   3. Optical properties:
      (a) Color: Color Harmony Manual No. 360
           St. Mellon 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, crenate, erose, ciliate, filamentous, curled.
      2. Internal structure - amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.
      c. Consistency - moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.
      d. Odor - Fecal
**Name of organism**: *Corynebacterium sp.*  
**Studied by**: Dr. W. B. Bollen  
**Culture No.**: 15A1  
**Source**: White Mountain  
**Habitat**: Soil  
**Date**: August 2, 1965

### CELL MORPHOLOGY

**Medium**: Trypticase soy agar  
**Temp.**: 25°C  
**Age**: 24 hr

#### Vegetative cells:
- Size: 0.5-0.8μm x 0.5-0.8μm
- Irregular forms:
- Sporangia: none, rare, spherical
- Bifid, oblong, ovoid, ovoidal, colonial,  
- **Position**: Central to eccentric, terminal, subterminal

#### Staining Characteristics
- **Gram**: +  
- **Age**: 24 hr  
- **Method**: Kopellof (modified)

### AGAR STROKE
- **Age**: 24 hr  
- **Temp.**: 25°C  
- **Amount of growth**: scanty, moderate, abundant  
- **Form**: uniform, circular, filamentous, rhizoid, irregular  
- **Elevation**: effuse, flat, raised, convex  
- **Surface**: smooth, wavy, confluent, concentric, rugose  
- **Margin**: entire, undulate, crenate, filamentous, curved  
- **Density**: compact, translucent

### AGAR COLONIES
- **Age**: 3 day  
- **Temp.**: 25°C  
- **Form**: punctiform, circular, filamentous, rhizoid, irregular

### NUTRIENT BROTH
- **Age**: 2 day  
- **Temp.**: 25°C  
- **Surface growth**: none, ring, andicule, facultative, membranous  
- **Subsurface growth**: none, turbid, granular  
- **Amount of growth**: scanty, moderate, abundant  
- **Sediment**: none, granular, facultative, revolute, fuffy

### GELATIN STAB
- **Age**: 7 day  
- **Temp.**: 25°C  
- **Liquefaction**: none, crescentiform, infundibulum, nopoliform, sacculate, stratiform  
- **Rate**: slow, moderate, rapid

### OTHER MEDIA
- **Age**:  
- **Temp.**:  
- **Potato slant**: 4gc Nude Tan Abundant  
- **Soybean Infusion agar**: 4ig Fawn Abundant  
- **Glucose nitrate agar**: White Scent  
- **Fat agar**: 3ea Pearl Pink Abundant

### ACTION ON MILK
- **Temp.**: 25°C  
- **Indicator**: Litmus  
- **Days**: 4  
- **Reaction**: ALKALINE  
- **Acid curd**:  
- **Rennet curd**:  
- **Peptonization**:  
- **Reduction (before coagulation)**:  

---

*Soil diptheroid; no described species.  
Resembles closely 15A.*
ACTION ON NITRATES

Medium: 1% KNO₃ Broth  Temp: 25 °C.
Nitrite: d: d; +3: d.
Gas (N): d: d; -3: d.

HYDROGEN SULFIDE PRODUCTION

Medium: Pb Acetate & H₂S: present, absent.
Age: 10 day  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 60°C: present, absent.

ACTION ON INDOLE

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

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose-nutrient agar  Age: 10 day  Temp: 28 °C.
Method: Shake tubes
Aerobic growth: absent, present, better than anaerobic growth, poorer than anaerobic growth.
Anaerobic growth: present, absent.
Uniform growth throughout

Pasteurization survival, 80°C. 10 minutes: Negative

ADDITIONAL TESTS

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

NH₄ from Peptone:

Metabolism:

Acetyl methyl carbinol:  Positive

NH₄ as sole Nitrogen source:

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

Methylen blue Reduction:

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

Cellulose Digestion:

Selenite-nutrient agar:  Positive
Cultural Characteristics of Bacterial Colonies

Culture No. 15A1

I. Surface Colonies. Age 8 days, on T5A medium.

a. Microscopic appearance.

1. Size, 3mm
2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.
   Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
   Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.
   Habit- compact, spreading.

3. Optical properties:
   (a) Color: Color Harmony Manual No. 3ea
   (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, filamentous, curled.
2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.
3. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.
4. Odor- cooking cabbage
Name of organism: Corynebacterium sp. Studied by Dr. W.B. Bollen. Culture No. 15B2.


**Cell Morphology**

Medium: Trypticase soy agar. Temp. 25 °C. Age: 24 hour.

Vegetative cells:
- Form and arrangement: streptococci, diplococci, microaerobic, aerobes, rods, cocci, spirals, branched rods, filaments.
- Mobility in broth: --
- Sign: --
- Colony forms: round, oval, irregular forms.
- Spore forms: none.
- Habitat: soil, decaying, intestinal, vascular, dramatic.
- Age: 24 hr.

**Staining Characteristics**


**Agar Stroke**

Age: 24 hr. Temp. 25 °C.

Amount of growth: scanty, moderate, abundant.
Form: falciform, echinulate, beaded, spreading, rhizoid.
Consistency: bumpy, viscous, membranous, brittle.
Chromogenesis: fluorescent, iridescent, photogenic.

**Agar Colonies**

Age: 8 day. Temp. 25 °C.

Form: punciform, circular, flabelliform, rhizoid, irregular.
Elevation: effuse, flat, raised, convex.
Surface: smooth, convoluted, raised, concave, rough.
Margin: entire, undulate, erose, flabelliform, curved.
Density: opaque, translucent.

**Nutrient Broth**

Age: 2 day. Temp. 25 °C.

Surface growth: none, ring, pellicle, asperulate, membranous.
Subsurface growth: none, turbid, granular.
Amount of growth: scanty, moderate, abundant.
Sediment: none, granular, asperulate, viscid, slaty.

**Gelatin Stab**

Age: 12 day. Temp. 25 °C.

Liquefaction: none, graniform, exudate, stabiform, napaiform, vestigial, stratiform.
Rate: slow, moderate, rapid.

**Other Media**

- Potato slant: No Growth
- Soybean Infusion agar: Scant
- Fat agar: Coral Red
- Glucose nitrate agar: No Growth

**Fermentation**


<table>
<thead>
<tr>
<th>Indicator</th>
<th>BCP</th>
<th>Acid in 2 °C days</th>
<th>Acid in °C days</th>
<th>Gas in 1 °C days</th>
<th>Gas in °C days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid curd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rennet curd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction (before coagulation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Action on Milk**

Temp. 25 °C.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus</td>
<td>2</td>
</tr>
<tr>
<td>Reaction</td>
<td>Very Slight</td>
</tr>
<tr>
<td>Acid curd</td>
<td>Alkaline</td>
</tr>
<tr>
<td>Rennet curd</td>
<td></td>
</tr>
<tr>
<td>Peptization</td>
<td></td>
</tr>
</tbody>
</table>

* Soil diphtheroid; no described species. Resembles 15B3 closely.
**ACTION ON NITRATES**

<table>
<thead>
<tr>
<th>Medium</th>
<th>KNO₃ broth</th>
<th>Temp. 28°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite (d)</td>
<td></td>
<td>-3 d</td>
</tr>
<tr>
<td>Gas (N) (d)</td>
<td></td>
<td>-3 d</td>
</tr>
</tbody>
</table>

**INDOLE PRODUCTION**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Tryptophane broth</th>
<th>Age: 10 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Kovac's</td>
<td>Temp. 28°C</td>
</tr>
<tr>
<td>Indole</td>
<td>present, absent.</td>
<td></td>
</tr>
</tbody>
</table>

**HYDROGEN SULFIDE PRODUCTION**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Acetate &amp; Thio-iron</th>
<th>Age: 10 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S</td>
<td>present, absent.</td>
<td></td>
</tr>
</tbody>
</table>

**TEMPERATURE RELATIONS**

<table>
<thead>
<tr>
<th>Age</th>
<th>Relation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>present, absent.</td>
</tr>
<tr>
<td>28°C</td>
<td>present, absent.</td>
</tr>
<tr>
<td>37°C</td>
<td>present, absent.</td>
</tr>
<tr>
<td>50°C</td>
<td>present, absent.</td>
</tr>
</tbody>
</table>

**PASTEURIZATION SURVIVAL, 80°C. 10 MINUTES:**

- **Negative**

**ADDITIONAL TESTS**

- **Casein Hydrolysis:** Negative
- **Fat Hydrolysis:** Negative
- **Gelatin Hydrolysis:** Negative
- **Starch Hydrolysis:** Negative
- **Urea Hydrolysis:** Negative
- **NH₄ from Peptone:** Negative
- **Metabolism:** Nonox.-nonferm.
- **Acetyl methyl carbinol:** Negative
- **NH₄ as sole Nitrogen source:** Positive
- **Sole Carbon sources:**
  - Citrate: Positive - slight
  - Glucose: Positive
  - Sucrose: Positive
  - Xylose: Positive - slight
- **Methylene blue Reduction:** Negative
- **Salt tolerances:**
  - 2%: Positive
  - 7%: Positive
  - 10%: Negative
- **Cellulose Digestion:** Negative
- **Selenite-nutrient agar:** Positive
Cultural Characteristics of Bacterial Colonies

Culture No. 1582

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

= 1. Size, 1 mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.
   Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
   Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.
   Habit- compact, spreading.

3. Optical properties:
   (a) Color: Color Harmony Manual No. 7 NC CHEEY
   (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, filamentous, curled.

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

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

d. Odor. Cooking cauliflower
**Name of organism**: Corynebacterium sp.  
**Studied by**: Dr. W.B. Bollen  
**Culture No.**: 1533  
**Source**: White Mountain  
**Habitat**: Soil  
**Date**: August 5, 1965

### Cell Morphology

<table>
<thead>
<tr>
<th>Medium</th>
<th>Trypticase soy agar</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative cells:</td>
<td>Age:</td>
<td></td>
</tr>
<tr>
<td>Form and arrangement: streptococci, diplococci, micrococci, sarcinae, rods, spirals, branched rods, filaments.</td>
<td>Age:</td>
<td></td>
</tr>
<tr>
<td>Mobility in broth:</td>
<td>Formed:</td>
<td></td>
</tr>
<tr>
<td>Endospores:</td>
<td>Shapes: spherical, ellipsoidal, cylindrical.</td>
<td></td>
</tr>
<tr>
<td>Position: central to eccentric, terminal, subterminal.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Staining Characteristics

<table>
<thead>
<tr>
<th>Gram:</th>
<th>+</th>
<th>Age: 24 hr</th>
<th>Method: Kopeloff (modified)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special stains:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Agar Stroke

<table>
<thead>
<tr>
<th>Age: 18 hour</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of growth: scanty, moderate, abundant.</td>
<td></td>
</tr>
<tr>
<td>Form: Aliform, coccoid, beaned, spreading, rhizoid.</td>
<td></td>
</tr>
<tr>
<td>Consistency: Brittle, rigid, membranous, brittle.</td>
<td></td>
</tr>
<tr>
<td>Chromogen: fluorescent, iridescent, photogenic.</td>
<td></td>
</tr>
</tbody>
</table>

### Agar Colonies

<table>
<thead>
<tr>
<th>Age: 8 day</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form: planctoniform, circular, flammulated, rhizoid, irregular.</td>
<td></td>
</tr>
<tr>
<td>Elevation: effuse, flat, raised, convex.</td>
<td></td>
</tr>
<tr>
<td>Surface: smooth, convexulated, radial, lemniscate, raggy.</td>
<td></td>
</tr>
<tr>
<td>Margin: entire, undulate, entire, flammulated, curved.</td>
<td></td>
</tr>
<tr>
<td>Density: sparse, translucent.</td>
<td></td>
</tr>
</tbody>
</table>

### Nutrient Broth

<table>
<thead>
<tr>
<th>Age: 3 day</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface growth: none, ring, puddle, flocculent, membranous.</td>
<td></td>
</tr>
<tr>
<td>Subsurface growth: none, fledgling, granular.</td>
<td></td>
</tr>
<tr>
<td>Amount of growth: scanty, moderate, abundant.</td>
<td></td>
</tr>
<tr>
<td>Sediment: none, granular, flocculent, rigid, flaky.</td>
<td></td>
</tr>
</tbody>
</table>

### Gelatin Stabs

<table>
<thead>
<tr>
<th>Age: 30 day</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquefaction: none, crateriform, in convexiform, non-fusiform, smooth, stratiform.</td>
<td></td>
</tr>
<tr>
<td>Rate: slow, moderate, rapid.</td>
<td></td>
</tr>
</tbody>
</table>

### Other Media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Potato dextrose broth</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid in 1C days</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Acid in 20 days</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

### Fermentation

<table>
<thead>
<tr>
<th>Medium</th>
<th>Nutrient broth</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate:</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>Indicators:</td>
<td>BCP</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Lactose</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Acid in</td>
<td>days</td>
<td>=</td>
</tr>
<tr>
<td>Gas in</td>
<td>days</td>
<td>=</td>
</tr>
</tbody>
</table>

### Action on Milk

<table>
<thead>
<tr>
<th>Indicator:</th>
<th>Litmus</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction:</td>
<td>Very slightly</td>
<td></td>
</tr>
<tr>
<td>Acid curd:</td>
<td>Alkaline</td>
<td></td>
</tr>
<tr>
<td>Rennet curd:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptonization:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction (before coagulation):</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Soil diptheroid; no described species.  
Resembles 15B2 closely.
ACTION ON NITRATES
Medium: 1% KNO₃ broth  Temp: 28°C

INDOLE PRODUCTION
Medium: Tryptophane broth Age: 10 day
Method: Kovac's
Indole: present, absent.

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Thio-iron Age: 10 day
H₂S: present, absent. Temp: 25°C

RELATION TO FREE OXYGEN - Catalase: Positive
Medium: Dextrose-nutrient agar Age: 10 day
Method: Shake tubes Temp: 25°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 (25°C.): present, absent.
Growth at 37°C.: present, absent.
Growth at 50°C.: present, absent.

Pasteurization survival, 80°C. 10 minutes: Negative

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

NH₄ from Peptone: Negative
Metabolism: Nonox.-nonferm.

Acetyl methyl carbinol: Positive

NH₄ as sole Nitrogen source

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

Methylene blue Reduction:

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

Cellulose Digestion: Negative
Selenite nutrient agar: Positive
Cultural Characteristics of Bacterial Colonies

Culture No. 1583

1. Surface Colonies. Age 8 days, on TSA medium.
   a. Microscopic appearance.

   = 1. **Size**: 2 mm

   2. **Shape**: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.
      Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
      Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.
      **Habit**: compact, spreading.

3. **Optical properties**:
   (a) **Color**: Color Harmony Manual No. 67 (Slame)
   (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, filamentous, curled.
   2. **Internal structure**: amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.
   c. **Consistency**: moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.
   d. **Odor**: cooking gard'flower
**Name of organism:** *Corynebacterium* sp.*<sup>†</sup>  
**Studied by:** Dr. W.B. Bollen  
**Culture No.:** 15  
**Source:** White Mountain  
**Habitat:** Soil  
**Date:** August 5, 1965

### CELL MORPHOLOGY

<table>
<thead>
<tr>
<th>Medium</th>
<th>Tryptic case soy agar</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vegetative cells:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Form and arrangement:</strong></td>
<td>streptococci, diplococci, micrococci, sarcinae, rods,</td>
<td></td>
</tr>
<tr>
<td><strong>Common:</strong></td>
<td>commas, spirals, branched rods, filaments.</td>
<td></td>
</tr>
<tr>
<td><strong>Mobility:</strong></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Size:</strong></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Spore:</strong></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Endospore:</strong></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Shape:</strong></td>
<td>spherical, ellipsoid, cylindrical.</td>
<td></td>
</tr>
<tr>
<td><strong>Position:</strong></td>
<td>central to eccentric, terminal, subterminal.</td>
<td></td>
</tr>
</tbody>
</table>

SEE PAGE 27 FOR PHOTOMICROGRAPHS

### STAINING CHARACTERISTICS

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

### AGAR STROKE

**Age:** 24 hr.  
**Temp.** 25 °C.

**Amount of growth:** scanty, moderate, abundant.  
**Form:** striiform, circular, filamentous, rhizoid, irregular.  
**Elevation:** flat, raised, convex.  
**Surface:** smooth, convoluted, radulate, concentric, rugose.  
**Margin:** entire, undulate, erose, filamentous, curved.  
**Density:** sparse, transluscent.

### AGAR COLONIES

**Age:** 8 day  
**Temp.** 25 °C.

**Form:** transverse, circular, filamentous, rhizoid, irregular.  
**Elevation:** flat, raised, convex.  
**Surface:** smooth, convoluted, radulate, concentric, rugose.  
**Margin:** entire, undulate, erose, filamentous, curved.  
**Density:** sparse, transluscent.

### NUTRIENT BROTH

**Age:** 2 day  
**Temp.** 25 °C.

**Surface growth:** none, ring, pellet, fusculent, membranous.  
**Subsurface growth:** none, turbid, granular.  
**Amount of growth:** scanty, moderate, abundant.  
**Sediment:** none, granular, fusculent, stolic.  
**Sediment:** none, granular, fusculent, stolic.

### GELATIN STAB

**Age:** 5 day  
**Temp.** 25 °C.

Liquefaction: none, enteriform, infundibuliform, napiform, sessile, transforn.

**Rate:** slow, moderate, rapid.

### OTHER MEDIA

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temp. °C.</th>
<th>Action on Milk Temp. °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato slant:</td>
<td>1 ½ ic</td>
<td>Lt. Antique Gold</td>
</tr>
<tr>
<td>Soybean Infusion agar:</td>
<td>3 ic</td>
<td>Camel</td>
</tr>
<tr>
<td>Fat Agar:</td>
<td>2 ea to 2 ea</td>
<td>Lt. Ivory to Lt. Wheat</td>
</tr>
<tr>
<td>Glucose Nitrate agar:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### FERMENTATION

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temp. 25 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbohydrate:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Indicator:</strong></td>
<td>BCP</td>
</tr>
<tr>
<td><strong>Glucose:</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>Lactose:</strong></td>
<td>=</td>
</tr>
<tr>
<td><strong>Sucrose:</strong></td>
<td>=</td>
</tr>
<tr>
<td><strong>Acid in 10 days:</strong></td>
<td>±</td>
</tr>
<tr>
<td><strong>Acid in days:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Gas in 10 days:</strong></td>
<td>=</td>
</tr>
<tr>
<td><strong>Gas in days:</strong></td>
<td>=</td>
</tr>
</tbody>
</table>

### ACTION ON MILK

<table>
<thead>
<tr>
<th>Indicator:</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus:</td>
<td>13, 14</td>
</tr>
<tr>
<td>Reaction:</td>
<td>neutral</td>
</tr>
<tr>
<td>Acid curd:</td>
<td></td>
</tr>
<tr>
<td>Rennet curd:</td>
<td></td>
</tr>
<tr>
<td>Peptonization:</td>
<td></td>
</tr>
<tr>
<td>Reduction (before coagulation):</td>
<td>±</td>
</tr>
</tbody>
</table>

* Soil diptheroid, no described species.
ACTION ON NITRATES
Medium: 1% KN03 broth Temp. 25° C.

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

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & H2S: present, absent.
Age: 10 day
Thiо-iron Temp. 28° C.
RELATION TO FREE OXYGEN
Catalase: Positive
Medium: Nutrient agar-Dextrose 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.

Growth in refrigerator (5°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: Negative

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

N14 from Peptone: Positive

Metabolism:

Acetyl methyl carbinol: Oxidizes Glucose, Xylose
Nonox.-nonferm. Sucrose, Lactose Negative

NH4 as sole Nitrogen source: Positive

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

Methylene blue Reduction: Positive

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

Cellulose Digestion: Negative

Selenite-nutrient agar: Positive
Cultural Characteristics of Bacterial Colonies

I. Surface Colonies. Age 8 days, on CSF2 medium.
   a. Microscopic appearance.

1. Size 3 mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.
   Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
   Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.
   Habit- compact, spreading.

3. Optical properties:
   (a) Color: Color Harmony Manual No. 2 6 13 B, AMBGG
   (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, filamentous, curled.

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

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

d. Odor. fecal
**Name of organism:** Corynebacterium sp.  
**Studied by:** Dr. W.B. Bollen  
**Culture No.:** 19E1  
**Source:** White Mountain  
**Habitat:** Soil  
**Date:** August 4, 1965

### CELL MORPHOLOGY

<table>
<thead>
<tr>
<th>Medium</th>
<th>Trypticase soy agar-temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative cells</td>
<td></td>
</tr>
<tr>
<td>Form and arrangement:</td>
<td>tryptococci, diphlococci, micrococci, sarcinae, rods, cocci, spirals, branched rods, filaments.</td>
</tr>
<tr>
<td>Mobility in broth:</td>
<td>Phagelastic: -</td>
</tr>
<tr>
<td>Size:</td>
<td>0.8 x 4.5 x 6.5 µm.</td>
</tr>
<tr>
<td>Sporangia:</td>
<td>heat, red, spiral, ellipsoidal, denote, traumatic.</td>
</tr>
<tr>
<td>Age:</td>
<td>24 hr.</td>
</tr>
<tr>
<td>Indicator:</td>
<td>b-----</td>
</tr>
</tbody>
</table>

### STAINING CHARACTERISTICS

| Gram:             | +  |
| Special stains:   | Age: 24 hr. Method: Kepeloff (modified) |

### AGAR STROKE

<table>
<thead>
<tr>
<th>Age: 24 hr.</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of growth:</td>
<td>scanty, moderate, abundant</td>
</tr>
<tr>
<td>Form:</td>
<td>Circular, filamentous, rhizoid, irregular</td>
</tr>
<tr>
<td>Consistency:</td>
<td>mucoid, mucoid, mucoid, mucoid, mucoid</td>
</tr>
<tr>
<td>Chromatogens:</td>
<td>Fluorescent, iridescent, photogenic</td>
</tr>
</tbody>
</table>

### AGAR COLONIES

<table>
<thead>
<tr>
<th>Age: 8 day</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form:</td>
<td>Punctiform, irregular, filamentous, rhizoid, irregular</td>
</tr>
<tr>
<td>Elevation:</td>
<td>Small, flat, raised, convex</td>
</tr>
<tr>
<td>Surface:</td>
<td>Reddish, rounded, red, concentric, rough</td>
</tr>
<tr>
<td>Margin:</td>
<td>Entire, wavy, entire, filamentous, curved</td>
</tr>
<tr>
<td>Density:</td>
<td>Sparse, translucent</td>
</tr>
</tbody>
</table>

### NUTRIENT BROTH

<table>
<thead>
<tr>
<th>Age: 2 day</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface growth:</td>
<td>none, ring, mollicule, focussate, membraneous</td>
</tr>
<tr>
<td>Subsurface growth:</td>
<td>none, turbid, granular</td>
</tr>
<tr>
<td>Amount of growth:</td>
<td>scanty, moderate, abundant</td>
</tr>
<tr>
<td>Sediment:</td>
<td>none, granular, focussate, rigid, bulky</td>
</tr>
</tbody>
</table>

### GELATIN STAB

<table>
<thead>
<tr>
<th>Age: 2 on</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquefaction:</td>
<td>none, wateriform, infundibuliform, napiform, succate, stratiform</td>
</tr>
<tr>
<td>Rate:</td>
<td>Slow, moderate, rapid</td>
</tr>
</tbody>
</table>

### OTHER MEDIA

<table>
<thead>
<tr>
<th>Age: 4 gc</th>
<th>Temp. °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato slant:</td>
<td>Nude Tan ab.</td>
</tr>
<tr>
<td>Soybean Infusion Agar:</td>
<td>Light Yellow to Pearl pink ab.</td>
</tr>
<tr>
<td>Fat Agar:</td>
<td>Light Yellow ab.</td>
</tr>
<tr>
<td>Glucose Nitrate Agar:</td>
<td>Scent.</td>
</tr>
</tbody>
</table>

### FERMENTATION

<table>
<thead>
<tr>
<th>Medium</th>
<th>Nutrient Broth</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate: 1% Glucose</td>
<td>Lactose</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Acid in 10 days</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Acid in 30 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas in 10 days</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Gas in 30 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### ACTION ON MILK

<table>
<thead>
<tr>
<th>Indicator:</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus: 4</td>
<td>Days</td>
</tr>
<tr>
<td>Reaction:</td>
<td>Alkaline</td>
</tr>
<tr>
<td>Acid curd</td>
<td></td>
</tr>
<tr>
<td>Rennet curd</td>
<td></td>
</tr>
<tr>
<td>Peptonization</td>
<td></td>
</tr>
<tr>
<td>Reduction (before coagulation)</td>
<td></td>
</tr>
</tbody>
</table>

* Soil diphtheroid; does not fit any described species.*
ACTION ON NITRATES
Medium: 1% KNO₃ broth       Temp 28 °C.

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

INDOLES PRODUCTION
Medium: K0-C

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate &        Age: 10 day
H₂S: present, absent. Thio-iron  Temp 28 °C.

RELATION TO FREE OXYGEN
Cat-lase: Positive
Medium: Dextrose-nutrient agar: 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
Aerobic growth: absent, poor.
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.

Growth at 37°C: present, absent.
Growth at 50°C: greater than anaerobic growth.

Pasteurization survival, 80°C. 10 minutes: Negative

ADDITIONAL TESTS

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

NH₄ from Peptone:

Metabolism:

Acetyl methyl carbinol:

NH₄ as sole Nitrogen source:

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

Methylene blue Reduction:

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

Cellulose Digestion:

Selenite-nutrient agar:

Positive - slight
Cultural Characteristics of Bacterial Colonies

Culture No. 1931

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

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

Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 30a.

(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, filamentous, curled.

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

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

4. Odor- Fecal.
Name of organism: *Corynebacterium* sp. * Studied by Dr. W. B. Bollen. Culture No. 1961
Source: White Mountain
Habitat: Soil
Date: August 5, 1965

### CELL MORPHOLOGY
**Medium:** Trypticase soy agar
**Temperature:** 25 °C
**Age:** 24 hr.

- **Vegetative cells:**
  - Form and arrangement: straight, pleomorphic, irregular, curved, rods
  - Motility in broth: Flagella
  - Spores: Present, regular forms
- **Endospores:** Absent, spherical, ellipsoidal, cylindrical

### STAINING CHARACTERISTICS
- **Gram:** +
- **Age:** 24 hr
- **Method:** Koepeff's (modified)
- **Special stains:**

### AGAR STROKE
**Age:** 24 hr.
**Temperature:** 25 °C

- **Amount of growth:** Scanty, moderate, abundant
- **Form:** Entire, granular, aerial, undulating, irregular
- **Consistency:** Liquid, viscous, membranous, slimy
- **Chromogens:** Absent, iridescent, photogenic

**4ea Lt. Apricot**

### AGAR COLONIES
**Age:** 7 day
**Temperature:** 25 °C

- **Form:** Punctiform, circular, filamentous, rhizoid, irregular
- **Elevation:** Flat, raised, convex
- **Surface:** Smooth, convex, radially, undulating, rough
- **Margin:** entire, undulate, even, filamentosus, curved
- **Density:** Opalescent, translucent

### NUTRIENT BROTH
**Age:** 2 day
**Temperature:** 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, faky

### GELATIN STAB
**Age:** 30 day
**Temperature:** 25 °C

- **Liquefaction:** None, crateriform, infundibuliform, nasiform, sauciform, crateriform
- **Rate:** Slow, moderate, rapid

### OTHER MEDIA
- **Potato slant:**
  - Age: 4gc
  - Temperature: Nude Tan
  - Amount: abundant
- **Soybean Infusion agar:**
  - Age: 4ea
  - Temperature: Flesh Pink
  - Amount: abundant
- **Glucose nitrate agar:**
  - Age: 3ea
  - Temperature: White
  - Amount: Pearl pink

### PERMUTATION
<table>
<thead>
<tr>
<th>Medium: Nutrient broth</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate: 1% BCP</td>
<td></td>
</tr>
<tr>
<td>Indicator: Litmus</td>
<td></td>
</tr>
<tr>
<td>Acid in 10 days</td>
<td>= = = =</td>
</tr>
<tr>
<td>Acid in days</td>
<td>= = = =</td>
</tr>
<tr>
<td>Gas in 10 days</td>
<td>= = = =</td>
</tr>
<tr>
<td>Gas in days</td>
<td>= = = =</td>
</tr>
</tbody>
</table>

### ACTION ON MILK
<table>
<thead>
<tr>
<th>Indicator: Litmus</th>
<th>Temp. 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid curd</td>
<td></td>
</tr>
<tr>
<td>Rennet curd</td>
<td></td>
</tr>
<tr>
<td>Peptization</td>
<td></td>
</tr>
<tr>
<td>Reduction (before coagulation)</td>
<td></td>
</tr>
</tbody>
</table>
### ACTION ON NITRATES

<table>
<thead>
<tr>
<th>Medium</th>
<th>Nitrite</th>
<th>Gas (N)</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% NaNO₃ broth</td>
<td>3</td>
<td>-3</td>
<td>28°C</td>
</tr>
</tbody>
</table>

### HYDROGEN SULFIDE PRODUCTION

<table>
<thead>
<tr>
<th>Medium</th>
<th>H₂S</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb Acetate &amp; Thio-iron</td>
<td>present</td>
<td>28°C</td>
</tr>
</tbody>
</table>

### INDOL PRODUCTION

<table>
<thead>
<tr>
<th>Medium</th>
<th>Age</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophane broth</td>
<td>10 day</td>
<td>28°C</td>
</tr>
</tbody>
</table>

### TEMPERATURE RELATIONS

<table>
<thead>
<tr>
<th>Condition</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth in refrigerator</td>
<td>present, absent</td>
</tr>
<tr>
<td>Growth at room temperature (28°C)</td>
<td>present, absent</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>present, absent</td>
</tr>
<tr>
<td>Growth at 50°C</td>
<td>present, absent</td>
</tr>
</tbody>
</table>

### Pasteurization survival, 80°C, 10 minutes:

Negative

### ADDITIONAL TESTS

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein Hydrolysis:</td>
<td>Negative</td>
</tr>
<tr>
<td>Fat Hydrolysis:</td>
<td>Negative</td>
</tr>
<tr>
<td>Gelatin Hydrolysis:</td>
<td>Negative</td>
</tr>
<tr>
<td>Starch Hydrolysis:</td>
<td>Negative</td>
</tr>
<tr>
<td>Urea Hydrolysis:</td>
<td>Positive</td>
</tr>
<tr>
<td>NH₄ from Peptone:</td>
<td>Negative</td>
</tr>
<tr>
<td>Metabolism:</td>
<td>Oxidizes Glucose, Sucrose</td>
</tr>
<tr>
<td>Acetyl methyl carbinol:</td>
<td>Negative</td>
</tr>
<tr>
<td>NH₄ as sole Nitrogen source:</td>
<td>Positive</td>
</tr>
<tr>
<td>Sole Carbon sources:</td>
<td>Positive</td>
</tr>
<tr>
<td>Citrate -</td>
<td>Positive</td>
</tr>
<tr>
<td>Glucose -</td>
<td>Positive</td>
</tr>
<tr>
<td>Sucrose -</td>
<td>Positive</td>
</tr>
<tr>
<td>Xylose -</td>
<td>Positive</td>
</tr>
<tr>
<td>Methylene blue reduction:</td>
<td>Positive</td>
</tr>
<tr>
<td>Salt tolerances:</td>
<td>Positive</td>
</tr>
<tr>
<td>2% -</td>
<td>Positive</td>
</tr>
<tr>
<td>7% -</td>
<td>Positive</td>
</tr>
<tr>
<td>10% -</td>
<td>Positive</td>
</tr>
<tr>
<td>Cellulose Digestion:</td>
<td>Negative</td>
</tr>
<tr>
<td>Selenite-nutrient agar:</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Cultural Characteristics of Bacterial Colonies

Culture No. 1901

I. Surface Colonies. Age 8 days, on TS medium.

a. Microscopic appearance.

1. Size, 2mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.
   Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
   Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.
   Habit- compact, spreading.

3. Optical properties:
   (a) Color: Color Harmony Manual No. 4ea Lt. Apricot
   (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, filamentous, curled.

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

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

d. Odor. Fecal
Name of organism: Corynebacterium sp. Studied by Dr. W. B. Bollem culture No. 19X

Source: White Mountain Habitat: Soil Date: August 5, 1965

CELL MORPHOLOGY
Medium: Trypticase soy agar Temp: 25°C.
Vegetative cells: Form and arrangement: streptococci, diplococci, micrococcus, sarcinae, rods, cocci, spores, branched rods, filaments.
Motility in broth: + Flagella: -
Size: 1-2.5 μm
Spores: none, rods, spindles, ellipsoidal, crenate, drumstick.
Endospores: Shape: spherical, ellipsoidal, cylindrical.
Position: central to eccentric, terminal, subterminal.

STAINING CHARACTERISTICS
Gram: + Age: 24 hr Method: Kopeloff (modified)
Special stains: 

AGAR STROKE
Age: 24 hr Temp: 25°C.
Amount of growth: agamy, moderate, abundant.
Form: diffusum, rhizoid, rounded, spreading, rhizoid.
Consistency: opaque, membranous, bristle.
Chromogenia: fluorescent; iridescent, phagedenic.

See Lt. Mellon Yellow

AGAR COLONIES
Age: 8 day Temp: 25°C.
Form: punciform, circular, filamentous, rhizoid, irregular.
Surface: smooth, crowded, raised, convex.
Surface: smooth, crowded, raised, convoluted, rough.
Margin: entire, undulate, erose, filamentous, curved.
Density: opaque, translucent.

NUTRIENT BROTH
Age: 2 day Temp: 25°C.
Surface growth: none, ring, pellicle, floculent, membranous.
Subsurface growth: none, liquid, granular.
Amount of growth: scant, moderate, abundant.
Sediment: none, granular, floculent, solid, foamy.

GELATIN STAB
Age: 12 day Temp: 25°C.
Liquefaction: none, crateriform, infundibulum, napiform, soviform, stelliform.
Rate: slow, moderate, rapid.

OTHER MEDIA
Age: 4 day Temp: 25°C.
Potato slant:
Fat agar:
Soybean Infusion agar:
Glucose nitrate agar:

FERMENTATION
Medium: Nutrient broth Carbohydrate: 1% Indicators: BCP

<table>
<thead>
<tr>
<th>Medium</th>
<th>Nutrient broth 1% Carbohydrate: BCP</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid in 2 days</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Acid in 6 days</td>
<td>2</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Gas in 2 days</td>
<td>2</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Gas in 24 days</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

ACtion ON MILK
Temp: 25°C

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus</td>
<td>2</td>
</tr>
</tbody>
</table>

Reaction: Alkaline.

Acid curd
Rennet curd
Peptonization
Reduction (before coagulation)

* Soil diptheroid.
ACTION ON NITRATES

Medium: \(1/5 \text{KNO}_3\) broth  Temp: 28°C.

Nitrite: \(d\)  Temp: 43°C.

Gas (N): \(-3\)  Temp: \(-3\)°C.

HYDROGEN SULFIDE PRODUCTION

Medium: Pb acetate & Thioc-iron  Age: 10 day  Temp: 28°C.

H2S: present, absent.

RELATION TO FREE OXYGEN

Medium: Dextrose-Nutrient agar  Age: 10 day

Method: Shake Tubes  Temp: 28°C.

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

Anaerobic growth: present, absent.

Pasteurization survival, 80°C, 10 minutes: Negative

ADDITIONAL TESTS

Casein Hydrolysis: Negative

Fat Hydrolysis: Negative

Gelatin Hydrolysis: Negative

Starch Hydrolysis: Negative

Urea Hydrolysis: Positive

NH4 from Peptone: Oxidizes Glucose, Sucrose

Metabolism: Nonferm. Lactose, Xylos

Acetyl methyl carbinol: Oxidizes Glucose, Sucrose

NH4 as sole Nitrogen source: Nonferm. Lactose, Xylos

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

Methylene blue Reduction:

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

Cellulose Digestion:

Selenite nutrient agar: Positive
Cultural Characteristics of Bacterial Colonies

Culture No. 19 X

I. Surface Colonies. Age 5 days, on TSA medium.

a. Microscopic appearance.

= 1. Size, 2 mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

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

Topography- smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3 en (lt. Mellow 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, crenate, erose, ciliate, filamentous, curled.

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

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

d. Odor. cooking cauliflower
**Name of organism**: Gorynebacterium sp. 
**Source**: White Mountain 
**Habitat**: Soil 
**Date**: August 6, 1965

### CELL MORPHOLOGY
- **Medium**: Trypticase soy agar
- **Temp**: 25 °C
- **Duration**: 18 hr
- **Form and arrangement**: pleomorphic, diplococci, micrococci, sarcinae, rods, cocci, spirilli, branched rods, filaments.
- **Morbidity in broth**: Fluid
- **Size**: 0.5 to 1.5 μm
- **Shape**: Coccioid
- **Position**: Centrally to eccentric, terminal, subterminal.

### STAINING CHARACTERISTICS
- **Gram**: +
- **Stain**: Modified Koppeloff

### AGAR STROKE
- **Temp**: 25 °C
- **Age**: 24 hr
- **Amount of growth**: Scant, moderate, abundant
- **Form**: Liquid, eckles, beaded, spreading, rhizoid.
- **Consistency**: Liquid, viscid, membranous, bristle.
- **Chromogenesis**: + fluorescent, iridescent, photogenic.

### AGAR COLONIES
- **Temp**: 25 °C
- **Age**: 24 hour
- **Shape**: Umbonate

### NUTRIENT BROTH
- **Temp**: 25 °C
- **Age**: 24 hr
- **Surface growth**: None, fine, pellicle, floculent, membranous
- **Subsurface growth**: None, turbid, granular
- **Amount of growth**: Scant, moderate, abundant
- **Sediment**: None, granular, floculent, mucid, frothy

### GELATIN STAB
- **Temp**: 25 °C
- **Age**: 30 days
- **Liquefaction**: None, wateriform, infundibuliform, nasiform, sacculose, transform
- **Rate**: Slow, moderate, rapid

### OTHER MEDIA
- **Potato slant**: 45a-45c Apricot-Nude Tan
- **Soybean Infusion agar**: White
- **Fat agar**: Pearl Pink
- **Glucose nitrate agar**: Scant

### FERMENTATION
- **Medium**: Nutrient broth
- **Indicator**: BCP
- **Temp**: 25 °C
- **Results**:
  - **Glucose**: +
  - **Lactose**: +
  - **Sucrose**: +

### ACTION ON MILK
- **Temp**: 25 °C
- **Indicator**: Litmus: 2 days
- **Reaction**: Neutral
- **Acid curd**: +
- **Rennet curd**: +
- **Peptonization**: +
- **Reduction (before coagulation)**: +

* Soil diptheroid.
### ACTION ON NITRATES

<table>
<thead>
<tr>
<th>Medium:</th>
<th>1% KN03 broth</th>
<th>Temp: 28°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite:</td>
<td>d.</td>
<td>-3. d.</td>
</tr>
<tr>
<td>Gas (N):</td>
<td>d.</td>
<td>-3. d.</td>
</tr>
</tbody>
</table>

### INDOLE PRODUCTION

<table>
<thead>
<tr>
<th>Medium:</th>
<th>Tryptophane broth</th>
<th>Age: 10 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>Kovac's</td>
<td>Temp. 28°C.</td>
</tr>
<tr>
<td>Indole:</td>
<td>present, absent.</td>
<td></td>
</tr>
</tbody>
</table>

### HYDROGEN SULFIDE PRODUCTION

<table>
<thead>
<tr>
<th>Medium:</th>
<th>Pb Acetate &amp; Thio-iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.S:</td>
<td>present, absent.</td>
</tr>
</tbody>
</table>

| Age: 10 day |
| 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.

### Pasteurization survival, 80°C. 10 minutes:

- Negative

### ADDITIONAL TESTS

- **Casein Hydrolysis:**
  - Negative
- **Fat Hydrolysis:**
  - Negative
- **Gelatin Hydrolysis:**
  - Negative
- **Starch Hydrolysis:**
  - Negative
- **Urea Hydrolysis:**
  - Positive
- **NH₄ from Peptone:**
  - Positive
- **Metabolism:**
  - Ferments Glucose, Sucrose
  - Nonox., nonferm. Lactose, Xylose
  - Negative
  - Positive
  - Positive
  - Positive - slight
  - Positive
  - Positive
  - Positive
  - Negative
  - Negative

### Methylene blue Reduction:

- Positive

### Salt tolerances:

- 2% -
- 7% -
- 10% -

### Cellulose Digestion:

- Negative

### Selenite-nutrient agar:

- Positive
Cultural Characteristics of Bacterial Colonies

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, 2mm

2. Shape: Outline - punctiform, circular, oval, irregular, filamentous, rhizoid.
   Elevation - effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.
   Topography - smooth, rough, wrinkled, contoured, stratified, concentrically ringed, radially ridged.
   Habit - compact, spreading.

3. Optical properties:
   (a) Color: Color Harmony Manual No. 3ca Pearl Pink
   (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, filamentous, curled.

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

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

d. Odor - cooking cabbage
Name of organism: *Corynebacterium* sp.

Studied by: Dr. W.B. Bollen

Culture No.: 122B

Source: 

Habitat: Soil

Date: August 6, 1965

### CELL MORPHOLOGY

**Medium:** Trypticase soy agar, temp. 25 ºC.

- **Vegetative cells:**
  - Form and arrangement: straight, diplococci, microcolonies, and chains.
  - Shape: rod, spiral, and filaments.
  - Motility: negative.
  - Sporangia: none.
  - REDUCTION (spherical, ellipsoidal, cylindrical).
  - Position: central to eccentric, terminal, subterminal.

**SEE PAGE 47 FOR PHOTOMICROGRAPHS**

### STAINING CHARACTERISTICS

- **Gram:** +
- **Age:** 24 hr.
- **Method:** Kopeloff (modified)
- **Special stains:**

### AGAR STRORE

- **Age:** 24 hr.
- **Temp:** 25 ºC.

- **Amount of growth:** dense, moderate, abundant.
- **Form:** diffuse, circular, filamentous, rhizoid, irregular.
- **Elevation:** flat, raised, meniscus.
- **Surface:** smooth, chelated, radiate, concentric, rugose.
- **Margin:** regular, undulate, crenate, filiform, curved.
- **Density:** opaque, translucent.

### AGAR COLONIES

- **Age:** 8 day
- **Temp:** 25 ºC.

### NUTRIENT BROTH

- **Age:** 1-9 days.
- **Temp:** 25 ºC.

### GLATIN STAB

- **Age:** 12 day +
- **Temp:** 25 ºC.

### OTHER MEDIA

- **Potato slant:**
  - 4gco
  - Nude Tan
  - Abundant

- **Soybean Infusion agar:**
  - 30a
  - Pearl Pink
  - Abundant

- **Glucose nitrate agar:**
  - 30a
  - White
  - Abundant

- **Fat agar:**
  - Pearl Pink
  - Abundant

<table>
<thead>
<tr>
<th>PERMUTATION</th>
<th>Temp. 25 ºC.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medium:</strong> Nutrient broth</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate: BCP</td>
<td></td>
</tr>
<tr>
<td>Indicator:</td>
<td></td>
</tr>
<tr>
<td>Acid in 10 days</td>
<td></td>
</tr>
<tr>
<td>Acid in days</td>
<td></td>
</tr>
<tr>
<td>Gas in 10 days</td>
<td></td>
</tr>
<tr>
<td>Gas in days</td>
<td></td>
</tr>
</tbody>
</table>

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* Soil diptheroid.
ACTION ON NITRATES
Medium: 1% KNO₃ broth  Temp. 28°C.
Nitrite: ...........d. ; ...........d. ; -3.....d.
Gas (N): ...........d. ; ...........d. ; -3.....d.

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Thio-iron  Age 10 day  Temp. 28°C.
H.S: 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: absent, absent.

PASTEURIZATION SURVIVAL, 80°C. 10 MINUTES: Negative

ADDITIONAL TESTS

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

NH₄ from Peptone:

Metabolism:

Acetyl methyl carbinol:

NH₄ as sole Nitrogen source:

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

Methylene blue reduction:

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

Cellulose Digestion:

Selenite-nutrient agar:

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

RELATION TO FREE OXYGEN
Catalase: Positive
Medium: Dextrose-nutrient agar  Age: 10 day  Temp. 28°C.
Method: Shake Tubes
Aerobic growth: absent, present, better than anaerobic growth, poorer than anaerobic growth.
Anaerobic growth: present, absent.
Cultural Characteristics of Bacterial Colonies

Culture No. 1228

I. **Surface Colonies.** Age 8 days, on TSA medium.

a. Microscopic appearance.

1. **Size:** 3mm

2. **Shape:** Outline- punctiform, **circular,** oval, irregular, filamentous, rhizoid.

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

**Topography:** smooth, rough, wrinkled, contoured, striated, concentrically ringed, radially ridged.

**Habit:** compact, spreading.

3. **Optical properties:**
   a. **Color:** Color Harmony Manual No. **3ea.** 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, crenate, erose, ciliate, filamentous, curled.

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

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

4. **Odor:** cooking cabbage