



Bacterial flora of the alimentary tract of *Grylloblatta campodeiformis campodeiformis* Walker
by Albert L Burroughs

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of master of Science in Bacteriology at Montana State College

Montana State University

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Abstract:

A study has been made of such bacteria as may be found in the alimentary tract of *Grylloblatta campodeiformis campodeiformis* Walker.

This insect is a cold-loving creature which lives at temperatures near freezing. With due precautions to avoid intruders, cultures were made from the alimentary tract by plating in dextrose agar. Incubations were made aerobically and anaerobically at 4°C, 18°C and 28°C. No growth was obtained anaerobically. Colonies from the aerobic plates were picked and streaked on dextrose agar. The optimum temperature for all but one culture was found to be room temperature (25°C). This one culture grew best at 17°C to 22°C.

Morphological studies of each of the cultures was made and inoculations were made into various media in an attempt to classify the strains of bacteria.

Fifty strains of organisms obtained from two specimens were included in this study. Sixteen (32 per cent) were found to be gram-positive cocci of the genus *Micrococcus*, 16 (32 per cent) were gram-positive rods of the genus *Bacterium*, and 18 (36 per cent) were gram-positive spore-formers of the genus *Bacillus*.

Five of the genus *Micrococcus* were classified to species. Three of these proved to be strains of *Micrococcus epidermidis* (Kligler) Hucker, one was variant of *Micrococcus subflavus* Bumm, and one a variant of *Micrococcus freudenreichii* Guillebeau. None of the genus *Bacterium* could be classified to species. Of the genus *Bacillus*, two were members of the *Aerobacillus* Group. Three strains were of the *Bacillus adhaerens* Group, one of which was possibly a variant of *Bacillus panis* Migula. One strain was a member of the *Bacillus circulans* Group and was possibly a variant of *Bacillus serusitidis* La Coste. One was a member of the Miscellaneous Group. The other eleven organisms were members of the *Bacillus subtilis* Group.

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CAMPODEIFORMIS CAMPODEIFORMIS WALKER

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ALBERT L. BURROUGHS

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ABS TRACT

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BACTERIAL FLORA OF THE ALIMENTARY TRACT OF GRYLLOBLATTA CAMPODEIFORMIS
CAMPODEIFORMIS WALKER

INTRODUCTION

This investigation was undertaken as an attempt to isolate and study the bacterial flora of the alimentary tract of Grylloblatta campodeiformis campodeiformis Walker. This insect was discovered in the Canadian Rockies in 1913 by Dr. E. M. Walker. Crampton (1915) has the following to say of the insect. "Grylloblatta campodeiformis combines within itself characters common to a number of 'Orthopteroid' insects. Indeed in many respects it may be considered as a veritable living fossil, and from the point of view of the study of insect phylogeny it is one of the most important of recent pterygotan forms."

Gurney (1937) states that the distributional data regarding Grylloblatta are still very fragmentary. The evidence points to the presence of many widely separated, more or less isolated, units of population. The three forms in the pacific coast states are distinct and the natural barriers clear. Grylloblatta sculleni occurs in Oregon. Grylloblatta campodeiformis occidentalis occurs on Mt. Baker, Washington. Grylloblatta barberi occurs in the Sierra Nevada Mountains in Plumas County, California, and is separated from its nearest Cascade relative by valleys in the watershed of the Klamath River. Grylloblatta campodeiformis campodeiformis occurs at Banff, Alberta and Gallatin County Montana. Another genus of the Grylloblattidae, Galloisiana occurs in Japan. This genus is represented by two subgenera, Galloisiana and Ishiana, nipponensis being the only species of Galloisiana and represented by one adult male and two nymphs. The

subgenus Ishiana is represented by one species, notabilis. A single male nymph has been taken of the latter.

Mills and Pepper, (1937) found that the temperature most desired by these insects was 3.7°C; that starting from 3.7°C, upon cooling there was a reduction in activity at 0.1°C and that this lethargic condition continued to -5.6°C. At this point there was increased activity for a short time, prostration ensuing at -6.2°C. Apparently the insect went into no dormant state before prostration. In travelling up the temperature scale from 3.7°C, the first change noted was an increase in activity at 15.5°C. There was partial paralysis of the legs at 24.9°C, quiescence at 26.4°C and final heat prostration at 27.8°C. This interesting work on the temperature relationships of this insect clearly demonstrates that a temperature which is compatible for most insects will produce death in the grylloblattid. It also shows the grylloblattid is very active at temperatures at which most insects would go into dormancy. Active grylloblattids are often collected from beneath objects which are frozen to the earth.

Indications from Walker's laboratory in Toronto are that the life cycle of the insect takes about 10 years. It is unusual in its development in that there is a pre-adult stage, the sub-imago. This is very unusual, the Ephemeridae or May-flies being the only other insects with such a stage. During the sub-imago the insect is white, lives in the ground and does not appear at the surface. After the last moult it assumes a honey yellow color. This information has not been published to date but was brought out in a conversation between Dr. H. B. Mills and Dr. E. M. Walker.

An insect which requires such a low temperature scale might possibly

be the source of a psychrophilic bacterial flora. With this in mind the investigation of the flora of the alimentary tract was begun.

Literature on the subject of insect-bacterial relationships is surprisingly limited. It would be supposed that, from the possibilities of biological control of insects, more investigations in this field would have been made; also that with regard to the importance of insects in the transmission of disease comparatively few species have been examined as to their bacterial flora. Steinhaus (1940) has recently surveyed the field and presents the best compilation of previous work done on this subject with regard to all phases of the biologic relationships existing between insects and the bacteria they harbor.

Leydig (1857) noted the presence of organisms in the caecal appendages of a pentatomid. Forbes in 1882 discovered numbers of bacteria in the caecae of certain Heteroptera.

Glasgow (1914) while working on the flora of the gastric caecae developed an aseptic method for removing the caecae as follows. "The insect is first lightly chloroformed to prevent struggling, the wings are clipped off near the base and the whole body moistened with alcohol to remove the film of air and allow the penetration of the bichloride solution which was usually used in the proportion of 1-500. The mercuric chloride solution is best applied with a bit of absorbent cotton held in a pair of old forceps. In this way the entire body of the insect can be thoroughly scrubbed with the disinfectant, so that any folds, such as those between the body segments, will certainly be moistened. After the bichloride solution has completely dried, which may be very well hastened by passing the insect

back and forth before a Bunsen flame, the flat edges of the abdomen are clipped off, from near the posterior end up to the thorax, with a pair of fine scissors which have been previously flamed. The top of the abdomen immediately back of the thorax may be cut across with sterile scissors and the resulting flap formed of the entire dorsal wall of the abdomen may then be lifted back with a pair of flamed forceps, leaving the abdominal viscera exposed."

Glasgow was able to get good bacterial smears from the caecae of certain species of Heteroptera, but could not obtain cultures on ordinary media. With other species he obtained luxuriant growth in nutrient broth. A squash vine infusion broth composed of nutrient broth and a decoction from 150 grams of squash vine per liter was successfully used in cultivating caecal organisms from the squash bug Anasa tristis. Glasgow found that the caecae of the Hemiptera of any one definite species invariably contained a pure culture of but one species of bacterium. The bacteria from different hosts varied greatly, from small coccus like organisms to large spiral forms. However, "in whatever form they occur they are morphologically characteristic for the particular species harboring them." Glasgow believed that these normal bacteria appear not only to inhibit the development of foreign bacteria but to exclude them altogether. This is a very interesting observation.

Pierce (1921) gives a summary of the records which have been made of the organisms isolated from cockroaches. Fifteen species of bacteria are listed as being either isolated from the feces of this insect, or the alimentary tract. Pierce also lists 53 species of bacteria that have been

isolated from the house-fly, some from the surface of the fly, and others from internal organs. Much of the bacteriological work done on the house-fly has been with abnormal flora. Attempts to experimentally introduce pathogens into the alimentary tract of the fly and recover the virulent pathogen from the feces have been numerous.

Steinhaus (1941) has made a survey of the bacterial flora of certain insects and finds "that most of the major types of bacteria are represented. These include gram-positive and gram-negative short rods, gram-positive spore-forming bacilli, and gram-positive cocci. The gram-negative short rods predominate, comprising slightly more than 50 per cent of the bacterial flora of the intestinal tracts of the insects studied." He also found "a number of bacteria which were elliptical in shape and which one is prompted to designate as coccobacilli. They varied in their physiologic characteristics as well as in their reaction to the gram-stain. These forms, however, may be pleomorphic types of the familiar short rods and cocci."

Altogether, Steinhaus made an investigation of the bacterial flora of 30 species of insects from 7 orders of the class Hexapoda. He isolated a total of 83 strains of bacteria, and made studies upon them in detail with regard to their morphologic, cultural, physiologic and pathogenic characteristics. He found that of the 83 strains 44 (53 per cent) were gram-negative short rods, 17 (20.5 per cent) were gram-positive spore formers. Steinhaus states, however, that "these percentages are given merely for the purpose of making approximate comparisons and not as the percentages of the various groups of bacteria as they may actually be found throughout the class Hexapoda." Also that these figures are subject to change depending on the interpretation

made of the data presented in his paper. Thirty-two of the strains studied did not conform to descriptions given in Bergey's manual. Of these, 27 were new strains and 5 were unidentified. From the 27 new strains, 21 new species were established.

Five species of Orthoptera were included in Steinhaus' investigation. Blattella germanica L., Neomibius fasciatus, var. fasciatus DeG., Conocephalus fasciatus, var. fasciatus DeG., Diapheromera femorata Say, and an unidentified member of the Tettigoniidae were the insects which composed this group. These yielded 19 strains of bacteria. Fifteen of these were strains of gram-negative short rods, 2 were strains of gram-positive cocci, and 2 were strains of gram-positive spore-formers.

EXPERIMENTAL METHODS

Living specimens of Grylloblatta campodeiformis campodeiformis were collected in the Fairy Lake region of the Bridger Range north of Bozeman, Montana. Upon bringing the specimens to the laboratory they were placed in the refrigerator so that they would survive until needed for the experiment. Two adult specimens were selected for dissection and treated as follows: The live insect was placed in a vial of 95 per cent alcohol for two to three minutes to remove the air film and allow penetration of the disinfectant. Subsequently it was placed in a vial of 1-1,000 mercuric chloride for two to three minutes, then in a vial of sterile water and rinsed well. Next the insect was placed in a sterile petri dish with a beeswax layer in the bottom. It was dissected with alcohol-flamed instruments. A slit was made ventrally from the posterior part of the abdomen to the anterior part of the

thorax and the resulting flaps pinned to the sides, exposing the alimentary tract. The entire alimentary tract was then removed and divided into fore-gut, mid-gut, and hind-gut. Each portion was placed in a sterile 10 cc. water blank and emulsified with a sterile rod. The emulsified portions and water were then distributed among a number of petri dishes and the dishes poured with dextrose agar. Both aerobic and anaerobic plates of each portion of the tract were incubated at 4°C, 18°C, and 28°C. After three days the plates were examined and it was found that all anaerobic plates were devoid of growth. The aerobic plates incubated at 28°C had copious growth and those at 18°C fair growth. After a few weeks of further incubation a few colonies appeared in the plates being incubated at 4°C. Colonies were picked from all plates, with an attempt to make a representative selection of the organisms present, and streaks were made on dextrose agar slants. After growth was obtained on the slants each culture was replated to further insure pure cultures. The optimum temperature for all but one of the cultures was found to be room temperature (25°C). One culture grew best at 17°C to 22°C.

From the morphological studies made on these cultures it became apparent that a diverse group of organisms was present, and that within the limitations of this paper it would be wise to attempt merely an identification of the organisms from the two individuals and to make no attempt to determine whether this flora is uniform in a large number of specimens.

Morphological studies were made in detail and much trouble was experienced due to pleomorphism. Inoculations were made into various

media and here again strain variation became evident. With few exceptions all inoculations were made in duplicate and often in triplicate or quadruplicate.

Incubations were made at room temperature except with gelatin, which was incubated in the 20°C incubator. Regarding incubation time, starch hydrolysis tests were made after 5 to 7 days. Gelatin stabs were incubated 6 weeks. All other media were incubated for at least two weeks.

From time to time all cultures were examined for purity by replating and making gram-stains. At the close of this investigation all cultures included in this paper were again examined microscopically from 24 hour dextrose agar slants and the morphology checked with the data previously obtained for each culture.

A total of 90 strains of organisms was originally isolated from the two insects. Of these, 50 strains of Eubacteriales were finally included in this paper. Most of the others were eliminated as Actinomycetales, or of doubtful purity; several died after a few transfers on dextrose agar, on which they grew in varying degrees at first. Cultures from the first insect examined are labelled 'A'. Those from the second insect are labelled 'B'.

RESULTS

The fifty organisms isolated from the alimentary tract of Grylloblatta campodeiformis are listed below with the morphological and physiological characters found by the author. The results recorded were confirmed by repeated tests, and in those cases where they did not check the term variable was used.

A1 Micrococcus sp.

Cocci: 0.6 to 1.0 micron in diameter, occurring singly, in pairs, and in masses. Non-motile. Gram-positive.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, pinkish.

Litmus milk: Slowly becoming alkaline.

Hydrogen sulfide production slight.

Koser's citrate positive.

Starch hydrolysis positive.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose. Acid may or may not be produced from maltose. No acid from lactose, sucrose or mannitol.

Potato: Scanty, echinulate, raised, glistening, smooth, pink, medium not darkened.

Aerobic.

Habitat: Fore-gut.

A2 Micrococcus sp.

Cocci: 0.8 to 1.4 microns in diameter, occurring singly, in pairs and in masses. Gram-positive. Non-motile.

Gelatin stab: Napiform liquefaction.

Dextrose agar slant: Abundant, filiform, convex, glistening, smooth, creamy yellow.

Litmus milk: Slowly becoming weakly alkaline.

Hydrogen sulfide variable.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid in dextrose and maltose. No acid from sucrose, lactose or mannitol.

Potato: Scanty, echinulate, flat, glistening, smooth, yellow, medium unchanged.

Aerobic.

Habitat: Fore-gut.

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A3 Micrococcus sp.

Cocci: 1.1 to 1.4 microns in diameter, occurring singly, in pairs, and in masses. Non-motile. Gram-positive.

Gelatin stab: Stratiform, napiform or infundibuliform liquefaction.

Dextrose agar slant: Abundant, filiform, raised, glistening, smooth, yellow.

Litmus milk: Slowly becoming alkaline, reduced in bottom.

Hydrogen sulfide negative.

Koser's citrate positive.

Starch hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: Moderate, spreading, raised, dull, slightly verrucose, dull yellow, medium darkened.

Aerobic.

Habitat: Fore-gut.

A4 Micrococcus subflavus

Cocci: 0.8 to 1.6 microns in diameter, occurring singly, in pairs, and in masses. Non-motile. Gram-positive.

Gelatin stab: Liquefaction napiform or stratiform.

Dextrose agar slant: Abundant, filiform, convex, glistening, smooth, pale yellow.

Litmus milk: Slowly becoming alkaline.

Hydrogen sulfide not formed.

Koser's citrate positive.

Starch hydrolysis variable.

Nitrites not produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

No acid from dextrose, maltose, lactose, sucrose, or mannitol.

Potato: Scanty, filiform, convex, dull, smooth, yellow, medium unchanged.

Aerobic.

Habitat: Fore-gut.

A5 Micrococcus epidermidis

Cocci: 0.8 to 1.4 microns in diameter, occurring singly, in pairs and in masses. Gram-positive. Non-motile.

Gelatin stab: No liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, white.

Litmus milk: Slowly becoming acid. No curd.

Hydrogen sulfide not formed.

Koser's citrate variable.

Starch not hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Acid in lactose, sucrose, dextrose, and maltose, no acid in mannitol.

Potato: Scanty, echinulate, raised, dull, slightly rugose, gray, medium not darkened.

Aerobic.

Habitat: Fore-gut.

A6 Micrococcus epidermidis

Cocci: 0.4 to 0.8 micron in diameter, occurring singly, in pairs, and in masses. Gram-positive. Non-motile.

Gelatin stab: No liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, white.

Litmus milk: Slowly becoming acid. No coagulation.

Hydrogen sulfide not produced.

Koser's citrate negative.

Starch not hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose, maltose, sucrose and lactose. No acid from mannitol.

Potato: Scanty, echinulate, raised, glistening, smooth, grayish-white, medium not discolored.

Aerobic, facultative.

Habitat: Fore-gut.

A7 Micrococcus epidermidis

Cocci: 0.2 to 0.7 microns in diameter, occurring singly and in pairs.
Gram-positive. Non-motile.
Gelatin stab: No liquefaction.
Dextrose agar slant: Scanty, beaded, convex, glistening, smooth, white.
Litmus milk: Slowly becoming weakly acid.
Hydrogen sulfide not produced.
Koser's citrate positive.
Starch not hydrolyzed.
Nitrates reduced to nitrites.
Indol not produced.
Voges-Proskauer negative.
Acid from dextrose, sucrose, lactose, and maltose. No acid from mannitol.
Potato: Scanty, echinulate, raised, slightly dull, slightly verrucose, light gray, potato unchanged.
Aerobic.
Habitat: Fore-gut.

A8 Micrococcus sp.

Cocci: 0.3 to 0.4 micron in diameter, occurring singly and in pairs.
Gram-positive. Non-motile.
Gelatin stab: Stratiform liquefaction.
Dextrose agar slant: Moderate, echinulate, flat, glistening, smooth, grayish-brown.
Litmus milk: Reduction and curd in lower part. Peptonized in upper part.
Hydrogen sulfide not produced.
Koser's citrate negative.
Starch not hydrolyzed.
Nitrites produced from nitrates.
Indol not produced.
Voges-Proskauer negative.
Acid from lactose, sucrose, dextrose. No acid from mannitol.
Potato: Moderate, spreading, raised, glistening, smooth, gray.
Aerobic.
Habitat: Fore-gut.

B9 Micrococcus sp.

Cocci: 0.8 to 1.4 microns in diameter, occurring singly and in pairs and masses. Gram-positive. Non-motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, cream colored.

Litmus milk: Slowly becoming alkaline with reduction in bottom.

Hydrogen sulfide not produced.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Aerobic, facultative.

Habitat: Fore-gut.

Acid in dextrose, sucrose, lactose, maltose. No acid in mannitol.

A10 Micrococcus sp.

Cocci: 1.1 to 1.6 microns in diameter, occurring singly, in pairs and in masses. Non-motile. Gram-positive.

Gelatin stab: No liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, light orange.

Litmus milk: Slowly becoming alkaline.

Hydrogen sulfide not formed.

Koser's citrate variable.

Starch hydrolysis variable.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Potato: No growth.

Aerobic.

Weak acid produced from dextrose, lactose, sucrose, maltose. No acid from mannitol.

All Micrococcus sp.

Cocci: 0.6 to 1.0 micron in diameter occurring singly, in pairs and in masses. Non-motile. Gram-positive.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Moderate, beaded, raised, glistening, smooth, grayish-white.

Litmus milk: Slowly becoming alkaline, reduced in bottom.

Hydrogen sulfide not produced.

Koser's citrate positive.

Starch hydrolysis variable.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose, sucrose, maltose and lactose. No acid from mannitol.

Potato: Scanty, echinulate, flat, glistening, smooth, orange, medium unchanged.

Aerobic.

Habitat: Fore-gut.

B12 Micrococcus sp.

Cocci: 0.9 to 1.4 microns in diameter, occurring singly, in pairs and in masses. Gram-positive. Non-motile.

Gelatin stab: Napiform liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, dull, smooth, cream-colored.

Litmus milk: Slowly becoming weakly alkaline.

Hydrogen sulfide not produced.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose, sucrose, maltose, and lactose. No acid from mannitol.

Aerobic.

Habitat: Hind-gut

A13 Micrococcus sp.

Cocci: 0.2 to 0.7 micron in diameter, occurring singly, in pairs, and in masses. Gram-positive. Non-motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, transparent white.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide not produced.

Koser's citrate positive.

Starch hydrolysis slight.

Nitrates not reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Weak acid production from dextrose. No acid from maltose, lactose, sucrose, or mannitol.

Potato: Abundant, spreading, raised, glistening, smooth, dirty gray, medium darkened.

Aerobic.

Habitat: Fore-gut.

A14 Micrococcus sp.

Cocci: 0.7 to 1.5 microns in diameter, occurring in pairs. Non-motile. Gram-positive.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, echinulate, convex, glistening, smooth, grayish-white.

Litmus milk: Slowly becoming alkaline, reduced in bottom.

Hydrogen sulfide variable.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrites not produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

No acid in carbohydrates.

Potato: No growth.

Aerobic.

Habitat: Fore-gut.

Al5 Micrococcus sp.

Cocci: 0.3 to 1.2 microns in diameter, occurring singly and in masses.
Gram-positive. Non-motile.
Gelatin stab: Stratiform liquefaction.
Dextrose agar slant: Abundant, echinulate, raised, glistening, smooth, white.
Litmus milk: Slowly becoming acid. Reduced in lower two-thirds.
Soft curd on bottom.
Hydrogen sulfide not formed.
Koser's citrate positive.
Starch hydrolyzed slightly.
Nitrates not reduced.
Indol not produced.
Voges-Proskauer negative.
Acid in dextrose, lactose, sucrose, maltose, and mannitol.
Potato: Abundant, spreading, raised, glistening, smooth, dirty gray, medium darkened slightly.
Aerobic.
Habitat: Fore-gut.

Al6 Micrococcus freudenreichii

Cocci: 0.5 in diameter, occurring singly, in pairs, and in masses.
Non-motile. Gram-positive.
Gelatin stab: Infundibuliform liquefaction.
Dextrose agar slant: Scanty, filiform, smooth, raised, glistening, white.
Litmus milk: Slowly becoming acid.
Hydrogen sulfide negative.
Starch hydrolysis negative.
Koser's citrate positive.
Nitrates not reduced.
Indol not produced.
Voges-Proskauer negative.
Acid in dextrose, lactose, sucrose, maltose, and mannitol.
Potato: Abundant, spreading, raised, smooth, dull, grayish center, light orange edges, potato darkened.
Aerobic facultative.
Habitat: Fore-gut.

A17 Bacterium sp.

Short rods: Very pleomorphic. Some large and pear-shaped. 0.5 by 1.1 microns, occurring singly and in pairs. Asporogenous. Gram-positive. Non-motile.

Gelatin stab: No liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, grayish-white.

Litmus milk: Slowly becoming alkaline.

Hydrogen sulfide not produced.

Koser's citrate variable.

Starch not hydrolyzed.

Nitrate reduction variable.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose, lactose, maltose, sucrose, or mannitol.

Potato: Scanty, echinulate, raised, glistening, smooth, gray, medium unchanged.

Aerobic.

Habitat: Fore-gut.

A18 Bacterium sp.

Rods: Very pleomorphic. 0.8 to 1.6 microns, occurring singly, and in pairs. Gram-positive. Non-motile. Asporogenous.

Gelatin stab: No liquefaction.

Dextrose agar slant: Abundant, beaded, convex, glistening, smooth, cream-colored.

Litmus milk: Slowly becoming alkaline in upper portion, reduced below.

Hydrogen sulfide negative.

Koser's citrate variable.

Starch not hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose and sucrose. No acid in maltose, lactose, or mannitol.

Potato: Scanty, echinulate, raised, glistening, smooth, gray, medium not changed.

Aerobic.

Habitat: Fore-gut.

A19 Bacterium sp.

Rods: Pleomorphic. 0.3 micron wide by 1.1 to 1.7 microns long, occurring singly and in pairs. Gram-positive. Non-motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, filiform, raised, glistening, smooth, creamy-white color.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide negative.

Koser's citrate positive.

Starch hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Acid in dextrose and sucrose. No acid in lactose, maltose, or mannitol.

Potato: Abundant, spreading, raised, glistening, smooth, grayish-brown color, medium darkened.

Aerobic.

Habitat: Fore-gut.

B20 Bacterium sp.

Small rods: 0.3 to 0.5 micron wide by 0.8 to 1.5 microns long, occurring singly, in pairs and short chains. Gram-positive. Non-motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, white.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide negative.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrate reduction variable.

Indol not produced.

Voges-Proskauer negative.

Acid in dextrose and sucrose. No acid in lactose, maltose, or mannitol.

Potato: Abundant, spreading, raised, glistening, smooth, dirty gray, medium darkened.

Aerobic.

Habitat: Hind-gut.

B21 Bacterium sp.

Short rods: Pleomorphic. 0.7 to 0.8 micron wide by 0.8 to 1.7 microns long, occurring singly and in pairs. Gram-positive. Non-motile.

Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, smooth, creamy-white.

Litmus milk: Slowly becoming alkaline with reduction in bottom.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose and sucrose. Weak acid from mannitol. No acid in maltose, or lactose.

Potato: Abundant, spreading, raised, dull, smooth, dirty gray, medium darkened.

Aerobic.

Habitat: Fore-gut.

A22 Bacterium sp.

Short rods: 0.3 to 0.6 micron wide by 0.5 to 1.43 microns long, occurring singly and in pairs. Gram-positive. Non-motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, echinulate, raised, glistening, smooth, grayish-white.

Litmus milk: Slowly becoming peptonized in upper half of tube, reduced in lower portion with soft curd.

Hydrogen sulfide positive.

Koser's citrate positive.

Starch strongly hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid in dextrose, sucrose, lactose. No acid in maltose or mannitol.

Potato: Abundant, spreading, raised, dull, smooth, grayish-brown, medium darkened.

Aerobic.

Habitat: Fore-gut.

B23 Bacterium sp.

Small rods: 0.3 to 0.5 micron wide by 0.8 to 1.4 microns long, occurring singly and in pairs. Gram-positive. Non-motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, creamy white.

Litmus milk: Slowly becoming peptonized in upper portion. Soft curd and reduced below.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose. Acid production variable from sucrose. No acid produced from lactose, maltose, or mannitol.

Potato: Abundant, spreading, convex, smooth, glistening, dirty gray, medium unchanged.

Aerobic.

Habitat: Hind-gut.

A24 Bacterium sp.

Short rods: 0.84 to 1.12 microns wide by 1.4 to 1.5 microns long, occurring singly and in pairs. Gram-positive. Non-motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, white.

Litmus milk: Slowly becoming peptonized with reduction in bottom.

Hydrogen sulfide variable.

Koser's citrate positive.

Starch hydrolyzed.

Nitrates not reduced.

Indol not produced.

Voges-Proskauer negative.

No acid in mannitol, lactose, dextrose, maltose, or sucrose.

Potato: Abundant, spreading, raised, glistening, smooth, dirty gray medium darkened.

Aerobic.

Habitat: Fore-gut.

A25 Bacterium sp.

Short rods: 0.5 to 0.6 micron wide by 0.5 to 1.0 micron long, occurring singly and in pairs. Non-motile. Gram-positive. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, cream colored.

Litmus milk: Slowly becoming peptonized in upper portion, lower part reduced.

Hydrogen sulfide negative.

Koser's citrate positive.

Starch hydrolysis variable.

Nitrites not produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose and sucrose. No acid from maltose, lactose, or mannitol.

Potato: Abundant, spreading, raised, glistening, smooth, dirty gray, medium darkened.

Aerobic.

Habitat: Fore-gut.

B26 Bacterium sp.

Small rods: 0.3 to 0.7 micron wide by 1.1 to 1.5 microns long, occurring singly and in pairs. Gram-positive. Non-motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, smooth, creamy white.

Litmus milk: Slowly peptonized in upper portion with reduction below.

Hydrogen sulfide positive.

Koser's citrate positive.

Starch strongly hydrolyzed.

Nitrates not reduced.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose, lactose, sucrose, and mannitol. No acid from maltose.

Potato: Abundant, spreading, raised, glistening, smooth, dirty gray medium darkened.

Aerobic.

Habitat: Fore-gut.

A27 Bacterium sp.

Rods: Pleomorphic. 1.0 to 1.3 microns wide by 2.0 microns long, occurring singly, and in short chains. Gram-positive. Motile. Asporogenous.

Gelatin stab: Crateriform liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, grayish-white.

Litmus milk: Slowly becoming alkaline.

Hydrogen sulfide not produced.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrates not reduced.

Indol not produced.

Voges-Proskauer negative.

Weak acid from dextrose, sucrose, and maltose. No acid from lactose or mannitol.

Potato: Moderate, spreading, raised, glistening, smooth, gray, medium darkened.

Aerobic.

Habitat: Fore-gut.

A28 Bacterium sp.

Rods: 0.3 to 0.6 micron wide by 1.0 to 1.4 microns long, occurring singly and in pairs. Motile. Gram-positive. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, grayish-white.

Litmus milk: Slowly peptonized in upper three-quarters of tube, soft curd and reduced below. Duplicate alkaline in upper half, reduced in the lower half.

Hydrogen sulfide not formed.

Koser's citrate positive.

Starch hydrolyzed.

Nitrites not produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid produced in dextrose, sucrose, mannitol, and maltose. No acid in lactose.

Potato: Moderate, spreading, raised, dull, verrucose, yellow, medium darkened.

Aerobic.

Habitat: Fore-gut.

A29 Bacterium sp.

Rods: 0.2 to 0.5 micron wide by 1.4 to 2.2 microns long, occurring singly and in pairs. Motile. Gram-positive. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, dull yellow.

Litmus milk: Peptonization in upper two-thirds, lower part reduced and soft curd formed.

Hydrogen sulfide positive.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: Abundant, spreading, raised, glistening, smooth, dirty orange, medium darkened.

Aerobic, facultative.

Habitat: Fore-gut.

A30 Bacterium sp.

Rods: 0.2 to 0.3 micron wide by 0.9 to 1.5 microns long, occurring mostly singly, some in pairs. Gram-positive. Motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, dull yellow.

Litmus milk: Rapid peptonization finally throughout entire tube.

Hydrogen sulfide produced.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: Abundant, spreading, raised, glistening, smooth, dirty orange, darkened.

Aerobic, facultative.

Habitat: Fore-gut.

A31 Bacterium sp.

Small rods: 0.2 microns wide by 0.8 to 1.4 microns long, occurring singly. Gram-positive. Motile. Asporogenous.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Moderate, echimulate, raised, glistening, smooth, grayish-white.

Litmus milk: Slowly becoming alkaline, reduced in bottom.

Hydrogen sulfide negative.

Koser's citrate variable.

Starch not hydrolyzed.

Nitrates not reduced.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: Abundant, spreading, raised, glistening, smooth, grayish-brown, medium darkened.

Aerobic.

Habitat: Fore-gut.

A32 Bacterium sp.

Rods: 0.2 to 0.7 micron wide by 0.8 to 1.43 microns long, occurring singly and in pairs. Asporogenous. Gram-positive. Non-motile.

Gelatin stab: No liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth, pinkish.

Litmus milk: Inert.

Hydrogen sulfide not produced.

Koser's citrate negative.

Starch hydrolyzed.

Nitrites not produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid in dextrose, maltose, and lactose. No acid in sucrose or mannitol. No acid in raffinose.

Potato: Slight, beaded to scanty filiform, raised, glistening, smooth, yellow, medium not discolored.

Temperature relations: Grows at room temperature. Grows best at 17°C to 22°C.

Aerobic.

Habitat: Fore-gut.

A33 Aerobacillus Group

Large rods: 0.6 to 1.0 micron wide by 2.1 to 3.4 microns long, occurring singly, in pairs, and in short chains. Spores central, elongate. Sporangia cylindrical to slightly bulged. Motile. Gram-positive.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Moderate, beaded, convex, glistening, smooth, transparent white. Adherent to medium.

Litmus milk: Slowly becoming peptonized in upper one-third, lower part soft curd with indications of gas production.

Hydrogen sulfide negative.

Koser's citrate variable.

Starch hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer variable.

Acid and gas produced from lactose, dextrose, sucrose, maltose, xylose, l-arabinose, raffinose. Inert in mannitol.

Potato: Abundant, spreading, raised, glistening, dirty gray, medium darkened.

Aerobic, facultative.

A34 Aerobacillus Group

Medium rods: 0.3 to 0.6 micron in width by 2.8 to 3.6 microns long, occurring singly and in pairs. Spores central, elongate. Sporangia slightly bulged to cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, beaded, convex, glistening, verrucose, transparent white. Growth adherent to medium.

Litmus milk: Soft curd and reduced. Later becoming peptonized in upper half of tube, lower half hard curd with evidence of gas formation.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer variable.

Acid and gas from lactose, sucrose, dextrose, maltose, xylose, l-arabinose, and raffinose. No acid from mannitol.

Potato: Abundant, spreading, convex, glistening, rugose, gray. Medium not changed. Evidence of gas formation in bubbles at side of slant.

Aerobic, facultative.

A35 Bacillus adhaerens Group

Short rods: 0.3 to 0.7 microns wide by 0.8 to 1.4 microns long, occurring singly and in pairs. Spores central, small and round. Sporangia cylindrical. Gram-positive. Non-motile.

Gelatin stab: No liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, grayish-white. Non-adherent to medium.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide not produced.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

No acid in carbohydrates.

Potato: Moderate, spreading, raised, dull, rugose, pale grayish-orange, medium darkened.

Aerobic.

Habitat: Fore-gut.

B36 Bacillus adhaerens Group

Large rods: 0.84 to 1.4 microns wide by 2.9 to 4.3 microns long, occurring singly, in pairs, and short chains. Spores central, oval. Sporangia cylindrical. Gram-positive. Non-motile.

Gelatin stab: Stratiform liquefaction.

Agar slant: Abundant, spreading, raised, dull, rugose, gray, adherent to medium.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Weak acid production from dextrose, sucrose, and mannitol. No acid in maltose, lactose, l-arabinose, xylose, or raffinose.

Potato: Abundant, spreading, raised, glistening, smooth, dull orange, potato darkened.

Aerobic.

Habitat: Fore-gut.

A37 Bacillus adhaeren Group

Medium rods: 0.6 to 0.8 micron wide by 1.4 to 2.1 microns long, occurring singly and in pairs. Spores longer than broad, central. Sporangia cylindrical. Gram-positive. Non-motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, dull, rugose, dirty gray, non-adherent to medium.

Litmus milk: Peptonization commencing early and going to completion throughout the tube.

Hydrogen sulfide strongly produced.

Koser's citrate positive.

Starch hydrolysis variable.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer variable.

Acid from dextrose, sucrose, and mannitol. No acid from maltose, lactose, xylose, l-arabinose, or raffinose.

Potato: Abundant, spreading, raised, glistening, rugose, grayish-brown, potato darkened.

Aerobic to slightly facultative.

Habitat: Hind-gut.

B38 Bacillus circulans Group

Rods: 0.5 to 0.8 micron wide by 2.1 to 4.2 microns long, occurring singly and in pairs. Spores terminal to sub-terminal, longer than broad. Sporangia plectridia. Gram-positive. Motile.

Gelatin stab: Crateriform liquefaction.

Dextrose agar slant: Scanty, echinulate, raised, glistening, smooth, transparent gray, non-adherent to medium.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide production slight.

Koser's citrate negative.

Starch not hydrolyzed.

Nitrates not reduced.

Indol not produced.

Voges-Proskauer negative.

Acid not produced from carbohydrates.

Potato: Scanty, echinulate, raised, dull, verrucose, grayish-brown, medium darkened.

Aerobic.

Habitat: Hind-gut.

B39 Bacillus Miscellaneous Group

Rods: 0.3 to 0.6 micron wide by 1.2 to 2.8 microns long, occurring singly and in pairs. Spores central, small, slightly longer than broad. Sporangia slightly bulged. Gram-positive. Motile.

Gelatin stab: Napiform to stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, smooth, cream-colored, non-adherent to medium.

Litmus milk: Alkaline.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch not hydrolyzed.

Nitrates not reduced.

Indol produced.

Voges-Proskauer negative.

Acid produced from dextrose, l-arabinose, xylose, and weakly from mannitol. No acid produced from sucrose, lactose, maltose, or raffinose.

Potato: Moderate, spreading, raised, glistening, rugose, reddish-brown growth, darkening of medium.

Aerobic.

B40 Bacillus subtilis Group

Medium to large rods: 0.7 to 1.1 microns wide by 2.4 to 4.3 microns long, occurring singly, in pairs, and in short chains. Spores central, ovoid. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, rugose, dirty-gray, adherent to medium.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch strongly hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Weak acid production from mannitol. No acid produced from dextrose, lactose, maltose, sucrose, xylose, l-arabinose, or raffinose.

Potato: Abundant, spreading, raised, glistening becoming dull, smooth, dull orange, medium darkened.

Aerobic.

Habitat: Fore-gut.

B41 Bacillus subtilis Group

Large rods: 0.9 to 1.1 micron wide by 1.6 to 5.7 microns long, occurring singly, in pairs, and short chains. Spores central, oval and large. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, glistening, smooth to slightly verrucose, grayish-white, non-adherent to medium.

Litmus milk: Slowly becoming peptonized with reduction on the bottom.

Hydrogen sulfide produced.

Koser's citrate positive.

Starch slightly hydrolyzed.

Nitrites not produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: Abundant, spreading, raised, dull, smooth, dull orange, medium darkened.

Aerobic.

Habitat: Hind-gut.

B42 Bacillus subtilis Group

Large rods: 0.7 to 0.8 micron wide by 1.7 to 5.7 microns long, occurring singly, in pairs, and in short and long chains. Spores central to sub-central, oval. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, smooth, gray, non-adherent to medium.

Litmus milk: Slowly becoming peptonized.

Hydrogen sulfide produced.

Koser's citrate positive.

Starch hydrolyzed slightly.

Nitrates not reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: No growth.

Aerobic.

Habitat: Hind-gut.

B43 Bacillus subtilis Group

Large rods: 1.4 to 1.5 microns wide by 4.6 to 5.0 microns long, occurring singly, in pairs, and in short and long chains. Spores central, longer than broad. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, smooth to verrucose, grayish-white, non-adherent to medium.

Litmus milk: Slowly becoming peptonized above with reduction below.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch hydrolysis variable.

Nitrates not reduced.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: Abundant, spreading, raised, glistening, verrucose, dull orange, medium darkened.

Aerobic.

Habitat: Hind-gut.

B44 Bacillus subtilis Group

Large rods: 0.8 to 1.5 microns wide by 2.2 to 4.3 microns long, occurring singly, in pairs, and in long chains. Spores central, longer than broad. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, smooth to verrucose, grayish-white, non-adherent to medium.

Litmus milk: Slowly becoming peptonized with reduction in bottom of tube.

Hydrogen sulfide production variable.

Koser's citrate positive.

Starch slightly hydrolyzed.

Nitrates not reduced.

Indol not produced.

Voges-Proskauer negative.

No acid from carbohydrates.

Potato: Abundant, spreading, raised, dull, smooth, dirty orange.

Aerobic.

Habitat: Hind-gut.

A45 Bacillus subtilis Group

Medium rods: 1.2 to 1.4 microns wide by 2.2 to 2.9 microns long, occurring singly and in pairs. Gram-positive. Motile. Spores central, narrow, long. Sporangia cylindrical.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, dull, rugose, gray, adherent to medium.

Litmus milk: Becoming peptonized quite rapidly and finally going to completion throughout entire tube.

Hydrogen sulfide strongly produced.

Koser's citrate positive.

Starch strongly hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer variable.

Acid produced from dextrose and sucrose. Weak acid production in maltose and mannitol. No acid from lactose, xylose, l-arabinose, or raffinose.

Potato: Abundant, spreading, raised, dull, rugose, grayish-brown, medium darkened.

Aerobic, facultative.

Habitat: Hind-gut.

A46 Bacillus subtilis Group

Rods: 1.4 to 1.7 microns wide by 3.0 to 4.4 microns long, occurring singly, in pairs, and short chains. Spores central, longer than broad. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, flat, dull, slightly verrucose, gray, non-adherent to medium.

Litmus milk: Rapidly becoming reduced in bottom of tube with formation of a soft curd. Finally peptonized in upper two-thirds.

Hydrogen sulfide positive.

Koser's citrate positive.

Starch strongly hydrolyzed.

Nitrites formed from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid formed from dextrose, sucrose, lactose, and maltose. No acid in mannitol, xylose, l-arabinose, or raffinose.

Potato: Abundant, spreading, raised, dull, verrucose, cream-colored, medium slightly darkened.

Aerobic, facultative.

Habitat: Hind-gut.

A47 Bacillus subtilis Group

Large rods: 0.8 to 1.4 microns wide by 2.8 to 4.3 microns long, occurring singly and in pairs. Spores large and central, longer than broad. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, raised, dull, smooth, gray, non-adherent to medium.

Litmus milk: Peptonization commencing early and going to completion through-out tube.

Hydrogen sulfide produced.

Koser's citrate positive.

Starch hydrolysis strongly positive.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Acid from sucrose, dextrose, and maltose. No acid in lactose, mannitol, xylose, l-arabinose, or raffinose.

Potato: Abundant, spreading, convex, dull, verrucose, gray, medium not discolored.

Aerobic, facultative.

Habitat: Hind-gut.

B48 Bacillus subtilis Group

Medium rods: 0.84 to 1.1 microns wide by 2.2 to 2.9 microns long, occurring singly and in pairs. Spores central to sub-central, longer than broad. Sporangia with rounded ends, very slightly bulged. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Moderate, echinulate, raised, glistening, smooth, creamy white, non-adherent to medium.

Litmus milk: Slowly peptonized.

Hydrogen sulfide positive.

Koser's citrate positive.

Starch strongly hydrolyzed.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer variable.

Acid in dextrose, sucrose, xylose, and mannitol. No acid from lactose, maltose, l-arabinose, or raffinose.

Potato: Abundant, spreading, raised, dull, rugose, dirty gray, medium darkened.

Aerobic.

Habitat: Fore-gut.

A49 Bacillus subtilis Group

Medium rods: 1.1 to 1.4 microns wide by 2.6 to 2.9 microns long, occurring singly, in pairs and in short and long chains. Spores central, to sub-central, longer than broad. Sporangia slightly swollen, ends truncate. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, verrucose, transparent white, non-adherent to medium.

Litmus milk: Slowly becoming peptonized in upper one-third, reduced in lower portion.

Hydrogen sulfide slightly produced.

Koser's citrate positive.

Starch hydrolyzed.

Nitrates reduced to nitrites.

Indol not produced.

Voges-Proskauer negative.

Acid in dextrose, sucrose, and mannitol. No acid in lactose, xylose, maltose, l-arabinose, or raffinose.

Potato: Abundant, spreading, raised, glistening, smooth, creamy-gray, medium darkened.

Aerobic.

B50 Bacillus subtilis Group

Large rods: 1.1 to 1.4 microns wide by 2.86 to 4.3 microns long, occurring singly, in pairs and in short and long chains. Spores central, ovoid, large. Sporangia cylindrical. Gram-positive. Motile.

Gelatin stab: Stratiform liquefaction.

Dextrose agar slant: Abundant, spreading, convex, glistening, smooth, grayish-white, non-adherent to medium.

Litmus milk: Slowly becoming peptonized with reduction in the bottom.

Hydrogen sulfide produced.

Koser's citrate positive.

Starch hydrolysis slight to negative.

Nitrites produced from nitrates.

Indol not produced.

Voges-Proskauer negative.

Acid from dextrose, sucrose. No acid produced from lactose, maltose, mannitol, xylose, l-arabinose, or raffinose.

Potato: Abundant, spreading, raised, glistening, smooth, dull orange, medium darkened slightly.

Aerobic.

Habitat: Hind-gut.

TABLE I COCCI (GRAM-POSITIVE)

Strain Number	Motility	Gelatin	H ₂ S Production	Nitrates to Nitrites	Indol	Voges Proskauer	Koser's Citrate	Litmus Milk	Starch	Glucose	Lactose	Sucrose	Maltose	Mannitol	
A1	+	+	sl	+	-	-	+	B	+	A	-	-	V	-	<u>Micrococcus</u> sp.
A2	+	+	v	+	-	-	+	B	-	A	-	-	A	-	<u>Micrococcus</u> sp.
A3	+	+	-	+	-	-	+	BR	+	-	-	-	-	-	<u>Micrococcus</u> sp.
A4	+	+	-	-	-	-	+	B	v	-	-	-	-	-	<u>Micrococcus</u> subflavus
A5	-	-	-	+	-	-	v	A	-	A	A	A	A	-	<u>Micrococcus</u> epidermidis
A6	-	-	-	+	-	-	-	A	-	A	A	A	A	-	<u>Micrococcus</u> epidermidis
A7	-	-	-	+	-	-	+	slA	-	A	A	A	A	-	<u>Micrococcus</u> epidermidis
A8	-	+	-	+	-	-	-	pep/RC	-	A	A	A	-	-	<u>Micrococcus</u> sp.
B9	-	+	-	+	-	-	+	BR	-	A	A	A	A	-	<u>Micrococcus</u> sp.
A10	-	-	-	+	-	-	v	slB to i	v	A	A	A	A	-	<u>Micrococcus</u> sp.
A11	-	+	-	+	-	-	+	BR	v	A	A	A	A	-	<u>Micrococcus</u> sp.
B12	-	+	-	+	-	-	+	B	-	A	A	A	A	-	<u>Micrococcus</u> sp.
A13	-	+	-	-	-	-	+	pep	sl	A	-	-	-	-	<u>Micrococcus</u> sp.
A14	-	+	v	-	-	-	+	BR	-	-	-	-	-	-	<u>Micrococcus</u> sp.
A15	-	+	-	-	-	-	+	ARC	sl	A	A	A	A	A	<u>Micrococcus</u> sp.
A16	-	+	-	-	-	-	+	A	-	A	A	A	A	A	<u>Micrococcus</u> freudenreichii

TABLE II ASPOROGENOUS RODS (GRAM-NEGATIVE)

Strain Number	Motility	Gelatin	H ₂ S Production	Nitrates to Nitrites	Indol	Voges Proskauer	Koser's Citrate	Litmus Milk	Starch	Glucose	Lactose	Sucrose	Maltose	Mannitol	
A17	+	+	+	+	+	+	+	B	+	A	A	A	A	A	<u>Bacterium</u> sp.
A18	+	+	+	+	+	+	+	BR	+	A	+	A	+	+	<u>Bacterium</u> sp.
B19	+	+	+	+	+	+	+	pep	+	A	+	A	+	+	<u>Bacterium</u> sp.
B20	+	+	+	+	+	+	+	pep	+	A	+	A	+	+	<u>Bacterium</u> sp.
B21	+	+	+	+	+	+	+	B/R	+	A	+	A	+	A	<u>Bacterium</u> sp.
B22	+	+	+	+	+	+	+	pep/RC	+	A	A	A	+	+	<u>Bacterium</u> sp.
B23	+	+	+	+	+	+	+	p	+	A	+	V	+	+	<u>Bacterium</u> sp.
A24	+	+	+	+	+	+	+	pep/R	+	+	+	+	+	+	<u>Bacterium</u> sp.
A25	+	+	+	+	+	+	+	pep R	+	A	+	A	+	+	<u>Bacterium</u> sp.
B26	+	+	+	+	+	+	+	pep/R	+	A	A	A	+	A	<u>Bacterium</u> sp.
A27	+	+	+	+	+	+	+	B	+	A	+	A	A	+	<u>Bacterium</u> sp.
A28	+	+	+	+	+	+	+	pep/RC	+	A	+	A	A	A	<u>Bacterium</u> sp.
A29	+	+	+	+	+	+	+	pep/RC	+	+	+	+	+	+	<u>Bacterium</u> sp.
A30	+	+	+	+	+	+	+	pep	+	+	+	+	+	+	<u>Bacterium</u> sp.
A31	+	+	+	+	+	+	+	BR	+	+	+	+	+	+	<u>Bacterium</u> sp.
A32	+	+	+	+	+	+	+	i	+	A	A	+	A	+	<u>Bacterium</u> sp.

TABLE III SPORE-FORMERS (GRAM-POSITIVE)

Strain Number	Motility	Gelatin	H ₂ S Production	Nitrates to Nitrites	Indol	Voges-Proskauer	Koser's citrate	Litmus Milk	Starch	Glucose	Lactose	Sucrose	Maltose	Mannitol	Raffinose	Xylose	L-arabinose	
A33	+	+	-	+	-	+	+	pep/CG	+	AG	AG	AG	AG	-	AG	AG	AG	<u>Aerobacillus</u> Grp.
A34	+	+	+	+	-	+	+	pep/RCG	+	AG	AG	AG	AG	-	AG	AG	AG	<u>Aerobacillus</u> Grp.
A35	-	-	-	+	-	-	+	pep	-	-	-	-	-	-	-	-	-	<u>B. Adhaerens</u> Grp.
B36	-	+	+	+	-	-	+	pep	+	A	-	A	-	A	-	-	-	<u>B. Adhaerens</u> Grp.
A37	-	+	+	+	-	+	+	pep	+	A	-	A	-	A	-	-	-	<u>B. Adhaerens</u> Grp.
B38	+	+	sl	-	-	-	-	pep	-	-	-	-	-	-	-	-	-	<u>B. Circulans</u> Grp.
B39	+	+	+	-	+	-	+	B	-	A	-	-	-	A	-	A	A	<u>B. Misc. Group</u>
B40	+	+	+	+	-	-	+	pep	+	-	-	-	-	slA	-	-	-	<u>B. subtilis</u> Grp.
B41	+	+	+	-	-	-	+	pep/R	sl	-	-	-	-	-	-	-	-	<u>B. subtilis</u> Grp.
B42	+	+	+	-	-	-	+	pep	sl	-	-	-	-	-	-	-	-	<u>B. subtilis</u> Grp.
B43	+	+	+	-	-	-	+	pep/R	+	-	-	-	-	-	-	-	-	<u>B. subtilis</u> Grp.
B44	+	+	+	-	-	-	+	pep/R	sl	-	-	-	-	-	-	-	-	<u>B. subtilis</u> Grp.
A45	+	+	+	+	-	+	+	pep	+	A	-	A	A	A	-	-	-	<u>B. subtilis</u> Grp.
A46	+	+	+	+	-	-	+	RA/pep	+	A	A	A	A	-	-	-	-	<u>B. subtilis</u> Grp.
A47	+	+	+	+	-	-	+	pep	+	A	-	A	A	-	-	-	-	<u>B. subtilis</u> Grp.
B48	+	+	+	+	-	+	+	pep	+	A	-	A	-	A	-	A	-	<u>B. subtilis</u> Grp.
A49	+	+	sl	+	-	-	+	pep/R	+	A	-	A	-	A	-	-	-	<u>B. subtilis</u> Grp.
B50	+	+	+	+	-	-	+	pep/R	sl	A	-	A	-	-	-	-	-	<u>B. subtilis</u> Grp.

Legend--See Page 47

DISCUSSION

There is an obvious lack of gram-negative short rods in the bacterial flora of the alimentary tract of Grylloblatta campodeiformis as compared with the flora of other insects as found by Steinhaus (1941). Over 50 per cent of the bacterial flora of the insects examined by that worker was made up of gram-negative short rods. Steinhaus stated that he had given the percentages merely for the purpose of making approximate comparisons and that he did not intend that this data should be interpreted as the actual percentages of the various groups of bacteria found in insects in general.

A possible explanation of gram-negative organisms in the grylloblattid is to be found in the ecology of the insect, since in respect to habitat it differs so widely from other insects.

Steinhaus (1941) reported no gram-negative short rods from any of the species he studied of the orders Odonata, Homoptera, or Hymenoptera. However from the order Orthoptera (to which Grylloblatta campodeiformis belongs) he isolated gram-negative short rods from 4 species of the order, namely, Blattella germanica L., Neomibius fasciatus, var. fasciatus DeG., Conocephalus fasciatus, var. fasciatus DeG., and Diapheromera femorata, Say.

Of the 50 strains of bacteria included in this paper, 16 (32 per cent) were cocci of the genus Micrococcus, 16 (32 per cent) were gram-positive asporogenous rods of the genus Bacterium, and 18 (36 per cent) were gram-positive sporogenous rods of the genus Bacillus.

The morphologically distinct series of organisms found in this investigation appear to fall naturally into a series of closely related

groups. The members of these groups may differ slightly from one another but they appear more closely related both morphologically and physiologically to each other than they do to members of any other group. The various members of each group which differ to varying degrees with regard to morphology and physiological reactions are probably strains of the same species.

Of the cocci there appear to be 11 groups of strains, all of which belong to the genus Micrococcus. Strains A5, A6, and A7 appear to be slight variants of Micrococcus epidermidis (Kligler) Hacker. Strain A4 appears to be a variant of Micrococcus subflavus Bumm, strain A16 a variant of Micrococcus freudenreichii Guillebeau. None of the other strains of the Micrococci could be classified to species.

The 16 strains of gram-positive asporogenous rods appear to fall into 9 groups. All of these are placed in the genus Bacterium since they did not correspond with the description of any other genus, and they did not seriously violate the generic description of Bacterium, which is not, however, a sharply defined group.

Of 18 strains of the genus Bacillus there appear to be 8 groups of strains. Strains A33 and A34 are members of the Aerobacillus Group. Strains A35, B36, and A37 are members of the Bacillus adhaerens Group. A37 is possibly a variant of Bacillus panis Migula. B38 belongs to the Bacillus circulans Group and is possibly a variant of Bacillus serusitidis La Coste. B39 belongs to the Miscellaneous Group. B40, B41, B42, B43, and B44 are probably strains of a single species of the Bacillus subtilis Group. A45, A46, A47, B48, A49 and B50 are strains of another species of the

Bacillus subtilis Group.

Strains A17, A18, B19, B21, and A27 were very pleomorphic forms which according to some authors might be designated as coccobacillus. In some of these cultures there appeared large, pear-shaped organisms and rods, while in other cultures there appeared rods and cocci. As the cultures retained this characteristic after being replated five times it was decided to be pleomorphism rather than contamination. These cultures were placed in the genus Bacterium since there was a preponderance of definite rods in all instances.

No significance can be attached to the fact that the flora of the two specimens of insects indicated by 'A' strains and 'B' strains of organisms differ appreciably since, as previously stated, no attempt was made to determine whether the flora of the insect was constant.

Regarding the distribution of the organisms in the alimentary tract, a large majority of the cocci and non-sporulating rods were found in the anterior portion of the tract, whereas a majority of the sporulating rods were found in the posterior portion.

The 50 strains referred to represent a much smaller number of species. The number of species, would, no doubt, vary with the individual making the interpretation depending on how liberal or conservative he interpreted the data presented. Perhaps the most interesting observation made in this investigation is that so many different kinds of organisms can be isolated from two individuals of a single species of insect, so few of which can be identified as previously described species. Other investigators may well take issue of this report that 50 strains of organisms can be isolated from

any such source, so few of which can be definitely classified to species in Bergey's Manual. The fifth and latest edition of Bergey's Manual contains descriptions of 1335 species with a reference to the original place of publication of 5,600 descriptions. Probably no bacteriologist will question the opinion that there are very numerous species of undescribed bacteria. As has been previously stated investigation of the bacterial flora of insects are few, classification of organisms found even fewer. The various scattered reports regarding the bacterial flora of the alimentary tracts of insects exhibit a definite lack of attempts to accurately identify the organism studied, with the exceptions of Steinhaus (1940), White (1906), and very few others. Evidently the species found include many hitherto undescribed. Described species of bacteria isolated from insects are comparatively few in Bergey's Manual.

Little is known of the food habits of Grylloblatta campodeiformis. Mills and Pepper (1937) state, "Grylloblattids which were kept for observation were fed a diet of flies and white bread. They also fed on decaying organic matter gleaned from the moss or mulch in which they were kept. The flies which they were fed were killed before being placed with them. Live or even slightly moving flies excited the grylloblattids greatly, and they could not be induced to take other than completely motionless prey." If decaying organic matter is a part of the diet of the insect, or if it contaminates their food, one would expect a more varied bacterial flora in this insect than in phytophagous and juice-sucking insects.

The author does not intend that this paper shall be interpreted as a

complete study of the bacterial flora of Grylloblatta campodeiformis. To accomplish such a study a larger number of specimens of the insect would have to be examined. On the basis of 50 strains of organisms from two specimens of the insect one can readily see how the number of strains of organisms would increase with each additional specimen examined. This would increase the scope of the problem to such an extent that it would be a full time problem for a greater period of time than one normally spends acquiring a masters degree. An attempt would also have to be made to obtain a medium more suitable for the growth requirements of many of the strains which die after a few weeks on dextrose agar though transfers are frequently made.

SUMMARY

1. Fifty strains of bacteria were isolated from the alimentary tract of the orthopteran Grylloblatta campodeiformis campodeiformis Walker.

2. Morphological and physiological studies were made of these 50 strains with an attempt to identify each of them.

3. Of the 50 strains, 16 (32 per cent) were gram-positive cocci of the genus Micrococcus, 16 (32 per cent) were gram-positive asporogenous rods of the genus Bacterium, and 18 (36 per cent) were gram-positive sporogenous rods. Two of these latter strains were of the Aerobacillus Group, three of the Bacillus adhaerens Group, one of the Bacillus circulans Group, one of the Miscellaneous Group, and the remaining 11 strains comprised two species of the Bacillus subtilis Group.

4. Other interpretations may be made of the data included in this paper resulting in a somewhat different grouping of the organisms studied. The physiological characteristics upon which one would lay most stress is an important factor in determining whether 2 varying strains should be grouped together or not. How much a strain may vary from a described species and still be classified as that species is a very important factor also.

5. While the finding of previously undescribed species of bacteria in the intestinal tracts of insects is to be anticipated, judging from previous investigations, the number of such species isolated from Grylloblatta campodeiformis was found to be especially high.

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LEGEND FOR TABLES I, II, AND III
PAGES 37 - 38 - 39

A - - - - - Acid reaction
B - - - - - Alkaline reaction
C - - - - - Curd
G - - - - - Gas formation
i - - - - - inert
- - - - - negative
+ - - - - - positive
pep - - - - - peptonization
R - - - - - Reduced
sl- - - - - slight
v - - - - - variable
/ - - - - - data following / signifies reaction in
lower portion of tube

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