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# Occurrence of Coliforms, Fecal Coliforms, and Streptococci on Vegetation and Insects

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ABSTRACT

GELDREICH, E. E. (Division of Water Supply and Pollution Control, U.S. Public Health Service, Cincinnati, Ohio), B. A. KENNER, AND P. W. KABLER. Occurrence of coliforms, fecal coliforms, and streptococci on vegetation and insects. Appl. Microbiol. **12**:63–69. 1964.—This study considers the sanitary significance of coliforms, fecal coliforms, and streptococci isolated from 152 species of plants and 40 samples of insects. These specimens were collected from various ecological environments and grouped into several categories. Results indicate that typical coliforms of the warm-blooded animal gut contribute a relatively small percentage of the organisms associated with vegetation (14.1%) and insects, (14.9%). A total of 1,203 coliform strains from vegetation and 1,084 coliform strains from insects were classified as to IMViC type and fecal coliform. No type was predominant in either the vegetation or insect groupings. The biochemical results for 646 streptococci from vegetation and 226 cultures from insects were reported. The predominant group, *Streptococcus fecalis*, as defined by Sherman criteria, constituted a majority of all strains from vegetation and insects. The "Completed Coliform Test" is recommended for the examination of plant and insect specimens to eliminate the many anaerobic and aerobic sporeforming bacteria that frequently produce false positive reactions by the "Confirmed Test" procedure. These findings support the current interpretation of the significance of the fecal coliform test for stream investigations or for surface water quality evaluations.

The sanitary significance of a bacterial indicator of pollution is determined from accurate information on its probable sources and quantitative distribution in nature. Interpretation of this significance for the coliform group and the fecal coliform segment within the group has been, and currently is, a controversial subject. Much the same case can be made for the fecal streptococcus group. The coliform group and the fecal streptococcus group are composed of many species, of which some predominate in warm-blooded animal feces, and others may predominate in soil and on vegetation. During periods of rainfall, pollution indicators that might be associated with vegetation would enter the surface waters by way of storm water drainage.

Wilson et al. (1935) found the coliform content of grass, hay, and straw to be relatively small except on samples subjected to soil contamination. Thomas and McQuillin (1952) reported these microorganisms abundant in grass from both ungrazed herbage and intensively grazed pasture. Coliform bacteria were reported by Thomas and Hobson (1955) as normal inhabitants on ears and panicles of cereal crops. They also noted 2.7% of the 148 strains as capable of gas production at 44 C. Fraser et al. (1956) reported that coliform bacteria were seldom found on foliage of a wide variety of garden plants, trees and shrubs, and field plants. These authors stated that exceptions were a result of possible contamination by insects, animals, or dust. Many of the earlier investigations related to occurrence of coliform bacteria were based on temperatures other than 35 and 45 C and on media or methods lacking selectivity and sensitivity.

Sherman (1937) reported that *Streptococcus fecalis* and *S. liquefaciens* types were rather common on plants, but gave no further information. Mundt, Johnson, and Khatchikian (1958) examined leaves, flowers, and shoots of plants grown in cultivated and uninhabited areas for "enterococci" and coliform bacteria. They reported "the enterococci group" were isolated from 58.5% of 106 samples including 63 plant species, and coliform bacteria were isolated from 67.0% of these samples. In another study, Mundt (1961) reported "enterococci" from 27% of the flowers sampled and 6.8% of the buds from the same plants. Totals of 34, 32.2, and 10.4%, respectively, of the flowers from nonagricultural, agricultural, and grass plants contained "enterococci." Data reported on "enterococci" per gram of corn flowers indicated corn tassel samples contained Minimal Probable Number (MPN) densities to a maximum of 300,000 per g. Recently, Mundt (1963) described the occurrence of "enterococci" on plants from the Great Smoky Mountains. "Enterococci" were obtained from 14.2% of 2,169 flowers and 3.4% of 440 samples of leaves, buds, shoots, fruits, and seeds, but he found no evidence of plant-specific species or variants of the "enterococci."

These reports of pollution indicators on vegetation may, in part, be derived from insect contact. Steinhaus (1941) isolated 11 strains of coliform bacteria from the alimentary tract of two Orthoptera, one Hemiptera, one Coleoptera, and two Lepidoptera. *S. fecalis* was isolated in five insect species (one Orthoptera, one Hemiptera, one Homoptera, and two Lepidoptera). Fecal streptococci were reported by West (1951) to be present in the housefly (Musca

TABLE 1. *Percentile distribution by quartile of MPN values per gram for 152 vegetation and 40 insect samples*

| Sample classification | No. of samples | Confirmed | | | Completed | | | Fecal coliform | | | Fecal streptococci | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 25% | 50% | 75% | 25% | 50% | 75% | 25% | 50% | 75% | 25% | 50% | 75% |
| *Vegetation* | | | | | | | | | | | | | |
| Flowering heads, buds, blossoms | 81 | 0.2 | 109 | 27,800 | <2.0 | 4 | 177 | <0.2 | <2.0 | 0.5 | 0.5 | 27 | 790 |
| Foliage of: | | | | | | | | | | | | | |
| Garden vegetables | 27 | 18 | 1,090 | 5,420 | 0.9 | 43 | 330 | <2.0 | <2.0 | 0.2 | 2.0 | 35 | 490 |
| Ornamentals | 15 | 0.7 | 130 | 490 | 0.4 | 7.0 | 130 | <0.2 | <2.0 | 7.9 | <2.0 | 49 | 2,210 |
| Wild flowers | 11 | <0.2 | <2.0 | 490 | <0.2 | <2.0 | 2.2 | <0.2 | <0.2 | <0.2 | <0.2 | 33 | 109 |
| Sphagnum-type peat moss | 8 | <4 | <4 | <10 | <4 | <4 | <10 | <4 | <4 | <10 | <4 | <4 | <10 |
| Indoor-cultivated foliage | 10 | <0.2 | 0.5 | 2.3 | <0.2 | <0.2 | <2 | <0.2 | <0.2 | <0.2 | <0.2 | 0.2 | 0.8 |
| *Insects* | | | | | | | | | | | | | |
| Coleoptera (beetles) | 16 | 8,800 | 1,084,000,000 | 2,210,000,000 | 8,800 | 94,000,000 | 490,000,000 | <36 | <20 | 79,000 | 920,000 | 1,024,000,000 | 4,900,000,000 |
| Orthoptera (grasshoppers, etc.) | 11 | 172,000 | 330,000 | 34,000,000 | 1,200 | 33,000 | 9,400,000 | <20 | <20 | 230 | 70,000 | 790,000 | 3,300,000 |
| Hymenoptera (bees, wasps, and ants) | 7 | 120 | 1,090,000 | 27,800,000 | 80 | 42,600 | 27,800,000 | <20 | <20 | 140 | <20 | 49,000 | 240,000,000 |
| Diptera-Homoptera (flies-leafhoppers) | 3 | 1,300 | 3,200,000 | 15,800,000 | 1,300 | 852,000 | 15,800,000 | <20 | <40 | <40 | 700 | 344,000 | 3,200,000 |
| Lepidoptera (moths and butterflies) | 3 | 130 | 490 | 54,000,000,000 | <20 | <20 | 4,900,000,000 | <20 | <20 | <20 | 20 | 1,720 | 240,000 |

domestica). Eaves and Mundt (1960) recovered fecal streptococci from 20 of 26 species of insects.

The insufficient data available on the quantitative recovery of coliform, fecal coliform, and fecal streptococci from vegetation and insects indicated the need to apply such procedures as described in the investigations by Clark et al. (1957) and Geldreich et al. (1958, 1962*a*, *b*). A review of the possible distribution of these indicator microorganisms should include a study of the bacteria on vegetation and insects and a determination of the percentage of each type that are fecal coliforms or fecal streptococci. This determination would further evaluate the occurrence of these bacterial groups as indicators of fecal pollution.

## MATERIALS AND METHODS

A total of 152 species of plants were examined. These were grouped in the following categories: flowering heads, which included garden ornamental plants, field crops, and wild flowers; foliage from garden vegetables, field crops, wild flowers, trees, shrubs, and various grasses; sphagnum-type peat moss; and indoor-cultivated plants from greenhouses and homes. These specimens were collected from various ecological environments, including fields, woods, gardens, lawns, cultivated farmlands, railroad cuts, roadways, riverbanks, and marshlands within 30 miles of Cincinnati.

The 40 insect samples were collected from areas where the plants had grown, but not necessarily concurrently. The insect samples included sixteen Coleoptera, eleven Orthoptera, seven Hymenoptera, three Lepidoptera, two Diptera, and one Homoptera.

Samples of plant material in minimal amounts of 20 g were collected in disposable cups. Weighed portions of this material were added to 9 volumes of buffered sterile water and mixed in a blender at high speed for 30 sec. Insect specimens were collected in 1-g minimal amounts, diluted with 99 volumes of buffered sterile water, and then mixed in a blender. In either case, the suspension was permitted to settle for about 15 min and then given a final mixing at high speed for 15 sec.

The suspensions were examined for coliform bacteria by the "Confirmed" and "Completed" Tests with use of the multiple-tube procedures as described in *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, 1960). The fecal coliform test consisted of confirming all positive presumptive tubes in EC broth at 44.5 ± 0.5 C. MPN enumerations of the fecal streptococcus group were performed by the "Tentative Test" (American Public Health Association, 1960) with azide dextrose (AD) presumptive broth followed by confirmation of all positive tubes in ethyl violet azide (EVA) medium. All confirmed-positive EVA tubes were verified by a Gram stain preparation. Pour plates with KF streptococcus agar (Kenner, Clark, and Kabler, 1961) were made for the isolation of colonies to be studied in the subsequent species or group classification for types of streptococci present. All quantitative data were reported as number of organisms per gram of sample.

Possible inhibition of a fecal coliform by the suspensions of vegetation and insect samples was determined by placement of paper discs saturated with blender samples on pour plates containing a heavy concentration of an *Escherichia coli* strain. Each plate also contained several paper discs saturated with suitable antibiotics for positive controls. The pour plates were incubated 24 hr at 35 C and inspected for zones of growth inhibition.

A preliminary isolation of the coliform bacteria from the vegetation and insect samples was obtained by the use of the membrane filter procedure. A minimum of 20 to 50 isolated colonies with a typical sheen were transferred to phenol red lactose broth for fermentation tests and verified in brilliant green lactose broth. Streaking on Eosin Methylene Blue (EMB) Agar insured cultural purity. All purified strains were then inoculated into EC broth at 44.5 C and into the media necessary for identification and classification by IMViC types (American Public Health Association, 1960).

## RESULTS AND DISCUSSION

The results of the five-tube "Confirmed and Completed Test" and the fecal coliform and streptococcal group tests for each of the plant and insect groups are summarized in Table 1. No coliform or fecal coliform bacteria were detected from either indoor-cultivated foliage or sphagnum moss, and only 2.2 coliforms per g were detected from wild flower plants at the 75-percentile level by the "Completed Test." The upper-quartile values in the "Completed Test" for flower heads, foliage from garden vegetables, and ornamentals were, however, 177, 330, 130 coliforms

TABLE 2. *Occurrence of coliform types on vegetation*

| Coliform type | Flowers | | Foliage | | Summary | |
|---|---|---|---|---|---|---|
| | No. of strains examined | Per cent occurrence | No. of strains examined | Per cent occurrence | No. of strains examined | Per cent occurrence |
| ++−− | 59 | 8.4 | 69 | 13.7 | 128 | 10.6 |
| −−++ | 88 | 12.6 | 149 | 29.6 | 237 | 19.7 |
| −+−− | 0 | — | 23 | 4.6 | 23 | 1.9 |
| +++− | 1 | 0.1 | 1 | 0.2 | 2 | 0.2 |
| −+−+ | 101 | 14.5 | 67 | 13.3 | 168 | 14.0 |
| ++−+ | 96 | 13.7 | 20 | 4.0 | 116 | 9.6 |
| −+++ | 20 | 2.9 | 12 | 2.4 | 32 | 2.7 |
| ++++ | 168 | 24.0 | 123 | 24.4 | 291 | 24.2 |
| +−++ | 81 | 11.6 | 7 | 1.4 | 88 | 7.3 |
| −−−+ | 66 | 9.4 | 21 | 4.2 | 87 | 7.2 |
| −++− | 1 | 0.1 | 4 | 0.8 | 5 | 0.4 |
| −−+− | 12 | 1.7 | 7 | 1.4 | 19 | 1.6 |
| +−+− | 2 | 0.3 | 0 | — | 2 | 0.2 |
| +−−+ | 4 | 0.6 | 1 | 0.2 | 5 | 0.4 |
| +−−− | 0 | — | 0 | — | 0 | — |
| Total | 699 | — | 504 | — | 1,203 | — |
| EC positive | 76 | 10.9 | 93 | 18.5 | 169 | 14.1 |

per g, respectively. The fecal coliform test values at the 75-percentile level for these three groups were 0.5, 0.2, and 7.9 per g, respectively. A total of 790 streptococci per g of flower heads was detected at the 75-percentile level. Results from the foliage of garden vegetables, ornamentals, and wild flowers were 490, 2,210, and 109 fecal streptococci per g, respectively, at the upper-quartile level. At the same percentile level, only 0.8 streptococci per g were found from indoor-cultivated foliage, and less than 10 per g were detected from sphagnum moss.

Data at the 75-percentile level for various insect groups varied from 9.4 million to 4,900 million coliforms per g by the "Completed Test." Fecal coliforms at this level ranged from <20 to 79,000 per g. Fecal streptococcal results at this level varied from 0.24 million to 4,900 million per g.

Table 2 contains the results of the biochemical reactions for 1,203 coliform strains. These data represent 20 to 50 strains from each of 16 flower species and 14 foliage specimens. No IMViC type appears to predominate in either the flower or foliage samples. Fecal coliforms made up 10.9 and 18.5% of the flower and foliage coliform strains, respectively. Both vegetation sources had 13 different IMViC strains, of which 12 were common to both. A total of 76.4% of all coliforms found on flowers were represented by five types. Four types comprised 81% of all coliform strains examined from foliage specimens. The remaining coliform types for flowers and foliage occurred in varying percentages of less than 10%.

A compilation of IMViC types and fecal coliform composition for plants and insects is given in Table 3. Data on vegetation are a consolidation of the foliage and flower results of Table 2, since there was little difference in coliform type composition between the two groups. Note that in both classes of samples there is little contribution from those organisms, i.e., fecal coliforms, derived from warm-blooded animals. Specifically, the percentage of fecal coliform strains detected on vegetation and insects was 14.1 and 14.9, respectively.

No coliform type predominated in either the vegetation or insect classes. Five IMViC types in the vegetation class made up 78.1% of the coliform strains with the other 21.9% scattered among nine types. Among the insect coliforms examined, five types composed 87.7% of all IMViC strains. Five other strains contained the remaining 12.2%.

In this study, only 65 vegetation samples, or 42.8% of the 152 specimens examined, and 23 insect samples, or 57.5% of the 40 insect collections, contained sufficient streptococci to provide isolates for biochemical study. The results of these biochemical tests for the streptococci found on vegetation and insects are given in Tables 4 and 5, respectively. When streptococci were isolated, the predominant organisms grew at both 45 and 10 C, i.e., 87.8% of 646 streptococci from vegetation and 95.1% of the 226 strains from insects were in this category. No strains capable of growth at 45 but not at 10 C were found on insects. Among streptococci from vegetation, however, 8.8% or 57 strains were capable of growth at 45 but not at 10 C. Streptococci that grew at 10 but not at 45 C composed 3.4% of total strains isolated from vegetation and 4.8% of those from insects.

Results obtained in this study would suggest that typical coliforms of the warm-blooded animal gut, as measured by the fecal coliform test, contributed a relatively small percentage of the organisms associated with vegetation and insects. Fraser et al. (1956) suggested that most of the coliforms (which were *Aerobacter cloacae*) found on grains are of insect origin. Eaves and Mundt (1960) demonstrated that insects could transfer streptococci in the

TABLE 3. *Coliform distribution by IMViC types and elevated temperature (fecal) test*

| IMViC type | Vegetation coliform types | | | | Insect coliform types | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of strains examined | Percentage isolated | Positive EC tests | | No. of strains examined | Percentage isolated | Positive EC tests | |
| | | | No. of strains | Percentage positive | | | No. of strains | Percentage positive |
| ++−− | 128 | 10.6 | 120 | 93.8 | 134 | 12.4 | 129 | 96.3 |
| −−++ | 237 | 19.7 | 15 | 6.3 | 113 | 10.4 | 1 | 0.9 |
| −+−− | 23 | 1.9 | 11 | 47.8 | 0 | X | 0 | 0.0 |
| +++− | 2 | 0.2 | 1 | X | 0 | X | 0 | 0.0 |
| −+−+ | 168 | 14.0 | 4 | 2.4 | 332 | 30.6 | 27 | 8.1 |
| ++−+ | 116 | 9.6 | 3 | 2.6 | 118 | 10.9 | 5 | 4.2 |
| −+++ | 32 | 2.7 | 0 | 0.0 | 28 | 2.6 | 0 | 0.0 |
| ++++ | 291 | 24.2 | 0 | 0.0 | 254 | 23.4 | 0 | 0.0 |
| +−++ | 88 | 7.3 | 0 | 0.0 | 46 | 4.2 | 0 | 0.0 |
| −−−+ | 87 | 7.2 | 15 | 17.2 | 42 | 3.9 | 0 | 0.0 |
| −++− | 5 | 0.4 | 0 | 0.0 | 0 | X | 0 | 0.0 |
| −−+− | 19 | 1.6 | 0 | 0.0 | 0 | X | 0 | 0.0 |
| +−+− | 2 | 0.2 | 0 | 0.0 | 0 | X | 0 | 0.0 |
| +−−+ | 5 | 0.4 | 0 | 0.0 | 8 | 0.7 | 0 | 0.0 |
| +−−− | 0 | X* | 0 | 0.0 | 9 | 0.8 | 0 | 0.0 |
| Total | 1,203 | | 169 | 14.1 | 1,084 | | 162 | 14.9 |

* X − insufficient number of cultures studied.

environment to the flowers of various grasses, cereals, and nonagricultural plants. They also stated that pollution from wild animals was a remote possibility because of their very low populations in cultivated fields used in the experiments. Both groups believed that soil was not a major contributor, since soil samples surrounding the plants did not have the coliform or streptococcal composition found on plants.

Fraser et al. (1956) observed no definite predominant types on plants or on insects. Our data support this view and are in marked contrast to the results previously obtained for warm-blooded animals and soil. The similarity of IMViC types recovered from vegetation and insects suggests their intimate association. Mundt (1961) has suggested that "the enterococci" are temporary residents on plants and are disseminated among plants by several different agents, but principally by insects. Apparently, from strong circumstantial evidence, much the same case can be made for the coliform contribution. The low recoveries of fecal coliforms might imply an unsuitable environment on vegetation and insects. None, however, of the 152 vegetation or 40 insect samples exhibited an inhibitory effect on the fecal coliform strain used in the disc sensitivity test. Thus, the possibility of fecal coliform suppression appears untenable. Certainly the contribution from warm-blooded animal pollution is a minor factor in both vegetation and the dissemination agent, insects.

Quantitatively, the results in Table 1 indicate that warm-blooded animal contamination, as measured by the fecal coliform test, is usually not observed. The higher levels of streptococci reflect either their more frequent occurrence on vegetation or the inclusion of streptococcal types not of warm-blooded animal origin. Mundt et al. (1958) reported 58.5% of 106 plant samples contained "enterococci." One possible reason for this divergence of fecal coliform from streptococcal values may be found in an inspection of the MPN values for insects (Table 1). The high densities of streptococci and relatively low fecal coliform levels on insects are repeated in a similar pattern but at lower density levels on vegetation.

TABLE 4. *Biochemical reactions of 646 streptococci isolated from vegetation**

| No. of strains | Growth at | | Growth reaction in | | Reduction of | | Final pH in 1% glucose | Hydrolysis of starch† | Peptonization of litmus milk‡ |
|---|---|---|---|---|---|---|---|---|---|
| | 10 C | 45 C | 6.5% NaCl | pH 9.6 broth | Methylene blue (0.1%) | Potassium tellurite (1:2,500) | | | |
| 219 | + | + | + | + | + | + | 4.1–4.5 | 0 | 0 |
| 80 | + | + | + | + | + | + | 4.1–4.5 | 0 | + |
| 119 | + | + | + | + | + | + | 4.0–4.7 | + | 0 |
| 71 | + | + | + | + | + | + | 4.0–4.7 | + | + |
| 5 | + | + | + | + | + | + | 5.0–5.1 | 0 | 0 |
| 4 | + | + | + | + | 0 | + | 4.2–4.3 | 0 | 0 |
| 1 | + | + | + | + | 0 | + | 4.3 | 0 | + |
| 1 | + | + | + | + | 0 | 0 | 4.4 | 0 | 0 |
| 1 | + | + | + | + | + | 0 | 4.3 | 0 | 0 |
| 1 | + | + | + | + | 0 | + | 4.5 | + | 0 |
| 1 | + | + | + | 0 | + | + | 4.8 | + | 0 |
| 11 | + | + | 0 | + | + | 0 | 4.2–4.5 | 0 | 0 |
| 8 | + | + | 0 | + | + | + | 4.3–4.4 | 0 | 0 |
| 22 | + | + | 0 | + | + | + | 4.5–5.3 | + | 0 |
| 1 | + | + | 0 | + | + | + | 4.3 | + | + |
| 14 | + | + | 0 | 0 | + | + | 4.3–4.4 | 0 | 0 |
| 4 | + | + | 0 | 0 | + | 0 | 4.9–5.0 | 0 | 0 |
| 2 | + | + | 0 | 0 | 0 | 0 | 4.9 | 0 | 0 |
| 2 | + | + | 0 | + | 0 | 0 | 4.9–5.0 | + | 0 |
| 7 | 0 | + | + | + | + | + | 4.7–5.1 | 0 | 0 |
| 1 | 0 | + | + | + | + | + | 4.5 | + | 0 |
| 6 | 0 | + | 0 | + | + | + | 4.6–4.9 | 0 | 0 |
| 18 | 0 | + | 0 | + | + | + | 4.4–5.3 | + | 0 |
| 2 | 0 | + | 0 | + | + | + | 4.5 | + | + |
| 2 | 0 | + | 0 | 0 | + | + | 4.4–4.5 | + | 0 |
| 1 | 0 | + | 0 | 0 | + | 0 | 4.5–5.2 | + | 0 |
| 16 | 0 | + | 0 | 0 | + | 0 | 4.6–5.3 | 0 | 0 |
| 4 | 0 | + | 0 | 0 | 0 | 0 | 4.6–5.1 | 0 | 0 |
| 10 | + | 0 | + | + | + | + | 4.1–4.5 | 0 | 0 |
| 4 | + | 0 | + | + | 0 | + | 4.1 | 0 | 0 |
| 2 | + | 0 | 0 | + | + | + | 4.3 | 0 | 0 |
| 6 | + | 0 | 0 | 0 | + | + | 4.3–4.4 | 0 | 0 |

* All strains were catalase-negative; all strains grew in 40% bile broth.

† A total of 241 strains hydrolyzed starch (37.7%).

‡ A total of 155 strains peptonized litmus milk (24.0%).

TABLE 5. *Biochemical reactions of 226 streptococci isolated from insects**

| No. of strains | Growth at | | Growth reaction in | | Reduction of | | Final pH in 1% glucose | Hydrolysis of starch | Peptonization of litmus milk† |
|---|---|---|---|---|---|---|---|---|---|
| | 10 C | 45 C | 6.5% NaCl | pH 9.6 broth | Methylene blue (0.1%) | Potassium tellurite (1:2,500) | | | |
| 89 | + | + | + | + | + | + | 4.0–4.7 | 0 | 0 |
| 102 | + | + | + | + | + | + | 4.0–4.5 | 0 | + |
| 13 | + | + | + | + | 0 | + | 4.0–4.7 | 0 | 0 |
| 2 | + | + | + | + | + | + | 4.2 | 0 | 0‡ |
| 1 | + | + | + | + | 0 | + | 4.8 | 0 | + |
| 1 | + | + | + | + | + | 0 | 4.0 | 0 | 0 |
| 3 | + | + | + | 0 | + | + | 4.0–4.6 | 0 | 0 |
| 2 | + | + | 0 | + | 0 | + | 4.3–4.7 | 0 | 0 |
| 2 | + | + | 0 | + | 0 | 0 | 4.3–4.4 | 0 | 0 |
| 3 | + | 0 | + | + | + | + | 4.2–4.6 | 0 | 0 |
| 1 | + | 0 | + | + | + | 0 | 4.1 | 0 | 0 |
| 1 | + | 0 | 0 | + | 0 | 0 | 4.4 | 0 | 0 |
| 4 | + | 0 | 0 | + | 0 | + | 4.5–4.7 | 0 | 0 |
| 2 | + | 0 | + | + | 0 | + | 4.6 | 0 | 0 |

* All strains were catalase-negative; all grew in 40% bile broth.

† Of 226 strains, 103 (45.6%) peptonized milk.

‡ Arginine hydrolysis-negative, all other arginine tests performed positive with these exceptions.

It is possible that these high streptococci levels may result from some proliferation on insects. Mundt, Coggins, and Johnson (1962) demonstrated that *S. fecalis* var. *liquefaciens* can establish commensal growth on plants of beans, corn, rye, and cabbage. A similar adaptation to the insect environment could result in some multiplication of the streptococci and may account for the extremely high values found on the *Coleoptera* (beetles). Another factor that might have caused such high counts is in the streptococcus detection procedure. AD-EVA streptococcal medium will support the growth of *S. lactis*, as illustrated by Kenner et al. (1961). Stark and Sherman (1935) reported this organism from corn, navy beans, cabbage, wheat, garden peas, and head lettuce. *S. lactis* was also reported by West (1951) to be associated with the housefly, *Musca domestica*, and by Eaves and Mundt (1960), with various *Hymenoptera*, *Coleoptera*, and one species of *Neuroptera*.

It is of interest that, of the 646 strains isolated from vegetation, 241 (37.7%) hydrolyzed starch, even though all other criteria were typical of the "enterococcus group" as defined by Sherman (1937). These results concur with those of Langston and Bouma (1960) in a study of microorganisms of grass silage. They found that a majority of the streptococci with *S. fecalis* characteristics also hydrolyzed starch. In studies on fecal streptococci from water, warm-blooded animals, cold-blooded animals, insects, and soil, we have never found *S. fecalis* strains displaying this characteristic.

Peptonization of milk appears to be a relatively common characteristic of fecal streptococcal strains isolated from insects; 45.6% were positive, as opposed to 24.0% of strains from vegetation. In our study of 3,158 strains isolated from feces of man and warm-blooded animals, the results indicated 16.9% or 533 fecal streptococcal cultures peptonized milk. Since the percentage of peptonizing strains from insects is nearly three times that of strains from humans and other warm-blooded animal feces, the peptonizing characteristic may be important in studies of streptococcal strains from insects.

Data presented here indicate that though there are some coliform bacteria on many of the vegetation samples examined, they are fewer in numbers than reported in the literature. This discrepancy can be partly explained by the use of a "completed" coliform test to eliminate the many anaerobic and aerobic sporeforming bacteria, and by geographical differences in environment for the various studies reported. These sporeforming bacteria can be the cause of many false positive results in the brilliant green lactose broth confirmatory test. Such interferences were also observed in the "Confirmed" coliform test in a study of soil samples from various geographical areas. It is essential, therefore, to use a "Completed MPN Test" in all studies of coliform populations on vegetation.

These results confirm other reports that the numbers of coliforms, fecal coliforms, and fecal streptococci on plants are very low. They also show that the ratio of fecal coliforms to coliforms is quite small. It is apparent that significant numbers of these indicator organisms are not removed from vegetation by rainfall and carried away in the runoff. These findings support the current interpretation that fecal coliforms in surface waters are largely, if not completely, derived from fecal pollution of animal origin.

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