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# *Klebsiella* Biotypes Among Coliforms Isolated from Forest Environments and Farm Produce

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Samples of water, soil, needles, and bark were collected from three different forest environments and from a pulp and paper mill. In addition, samples of fresh produce were obtained from a local supermarket. All samples were examined for total and fecal coliforms. The counts obtained from the forest-related samples did not correlate with sample type or location. When 123 isolates were identified biochemically, 71% were *Klebsiella*, 19% *Enterobacter*, 8% *Citrobacter*, and 2% *Escherichia*. All the *Citrobacter*, 75% of the *Enterobacter*, and 65% of the *Klebsiella* were negative for growth in elevated coliform (EC) broth. All the *Escherichia* were EC positive. The counts obtained from the fresh produce were generally higher than the forest counts, but the distribution of biotypes was similar. Of the 146 isolates examined 64% were *Klebsiella*, 14% were *Escherichia*, 14% were *Enterobacter*, and 8% were *Citrobacter*. All the *Enterobacter* and *Citrobacter* were EC negative, whereas 25% of the *Klebesiella* and 80% of the *Escherichia* were EC positive.

As early as 1929, Edwards (8) noted the similarity in biochemical reactions between *Aerobacter aerogenes* and Friedlander's bacillus. When Ewing and Edwards (9) proposed a new system of classification for the *Enterobacteriaceae*, they eliminated the genus *Aerobacter* and replaced it with *Enterobacter* and *Klebsiella*. However, past literature refers to *Aerobacter* species as being frequent inhabitants of soil and vegetation. Thomas and McQuillin (23) classified at least 73% of the isolates obtained from grazed and ungrazed herbage as aerogenes-cloacae types, and less than 9% as *Escherichia coli*. Fraser et al. (11) found that the *Aerobacter* group was present on about 33% of the plants examined, although in their opinion the foliage of field and garden plants, (including trees and shrubs) rarely carried coli-aerogenes bacteria, except when contaminated by animals, insects, or dust.

Geldreich et al. (13) examined 152 samples of plants and 40 samples of insects and concluded that typical coliforms of the warm-blooded animal gut contributed a relatively small percentage of the organisms associated with vegetation and insects. They did not attempt to name the nonfecal coliforms they isolated but the indole, methyl red, Voges-Proskauer, citrate (IMViC) reactions listed suggest that more than 50% were *Aerobacter* types. Papavassiliou et al. (17) examined plant foliage and flowers collected in Greece, found that coli-aerogenes bacteria were seldom isolated from plants grown in uninhabited areas and concluded that they were not native flora. Vegetables and fruits sold in the market in Athens, however, were frequently contaminated with coli-aerogenes bacteria. Geldreich and Bordner (12) have reviewed the subject of fecal contamination of fruits and vegetables destined for market.

Nunez and Colmer (16) obtained 384 lactose-fermenting isolates from sugar cane and found that 88% of them were IMViC − − + + and would have been classified as *Aerobacter aerogenes* in the past. By using the classification of Ewing and Edwards (9), they found that 98% of the so-called *Aerobacter* isolates were *Klebsiella* and 2% were *Enterobacter*. Since the major differences between these genera are motility and the presence or absence of ornithine decarboxylase, a similar split might have occurred if these tests had been applied to the isolates of previous workers.

Water quality surveys have recently singled out pulp and paper mill wastes (3, 6, 18, 19, 22) as major sources of coliform group organisms. In many cases, this is despite the segregation of sanitary waste-treatment facilities. Past studies (14) have identified *A. aerogenes* as a major component of slime found in pulp and paper

mill process streams. However, by using the biochemical classification system proposed by Ewing and Edwards (9), the organisms isolated by Dutka et al. (6) have been identified as *Enterobacter*, *Klebsiella*, *Escherichia*, and *Citrobacter*, with *Klebsiella* being the dominant genus.

*Klebsiella* has been associated with upper respiratory tract and hospital-acquired infections, and the epidemiology of *Klebsiella pneumoniae* has recently been reviewed by Eickhoff (7). Martin et al. (15) have just published their findings from a 3-month study of its epidemiological significance. This investigation was prompted by the fact that a change in nomenclature has resulted in the routine isolation of potentially pathogenic *Klebsiella* from common environmental systems, which in the past were considered to be the habitat of saprophytic organisms known as *Aerobacter*.

The source or cause of the coliform counts on the fruit and vegetables examined during this study was not the object of this investigation. That subject has been adequately reviewed by Geldreich and Bordner (12). Rather it was the distribution of the coliform group biotypes which was of interest, and the routine exposure of the public to large numbers of such organisms.

## MATERIALS AND METHODS

**Source of samples.** One series of samples was taken from the Seymour watershed of the Greater Vancouver District, which is a virgin, coastal, evergreen forest, with strictly limited access. The samples of water, soil, bark, and needles were collected in November, 1970, from an area previously remote to humans. A second series of samples was collected from a forested portion of the Nanaimo watershed on Vancouver Island, to which public access has been severely restricted for the last 40 years. Samples of needles and bark were collected in May, 1971, from a public forested area (McMillan Grove Provincial Park on Vancouver Island) and from an integrated pulp and paper mill. Samples of bark were collected from standing trees, from logs hauled by truck from high in the mountains on Vancouver Island, and from logs that had been hauled by truck prior to dumping into the contaminated waters of Alberni Inlet. All samples were collected in an aseptic manner, stored in ice, and returned to the laboratory for testing within 18 hr.

Tomatoes and various vegetables were purchased in bulk from a local but nationally represented supermarket early on a Monday morning (August 1971) and returned to the laboratory immediately for processing. The origin, age, and handling procedures prior to purchase are unknown. The samples were homogenized in a Waring blendor at a 1:10 dilution in phosphate buffer (pH 7).

**Coliform tests.** The most-probable numbers (MPN) of total completed and fecal coliforms were determined by the multiple-tube technique (1). Positive tubes of Brilliant Green-bile-lactose broth and elevated coliform (EC) broth were streaked onto either eosin methylene blue (EMB) or MacConkey plates.

**Identification of isolates.** Colonies were picked from either EMB or MacConkey plates and transferred into lactose and EC broths. Lactose-positive cultures were transferred into triple sugar iron agar, sulfide indole motility medium, motility medium, methyl red-Voges-Proskauer medium, Simmon citrate agar, and ornithine decarboxylase medium. Incubation was at 35 C. The isolates were classified by comparing the reactions obtained with those listed by Ewing and Edwards (9) and Fife et al. (10), with emphasis being placed on results from the hydrogen sulfide, ornithine decarboxylase, motility, and citrate tests (HOMoC), as suggested by Closs and Digranes (4).

## RESULTS

**Coliform counts.** The total completed and fecal coliform counts, expressed as MPN/100 g (wet weight), from the forest samples, are listed in Table 1. All samples of water and soil examined gave a positive coliform count with the highest count recorded being 4,300. However, only one water sample gave a positive fecal coliform count. Positive coliform counts were obtained for all moss, fern, and needle samples taken from the Seymour watershed, whereas hemlock and Douglas fir needle samples taken from the Nanaimo watershed did not have a detectable count. The highest count, 54,000, was obtained from cedar needles taken from the Nanaimo watershed. Positive fecal coliform counts were obtained from the fern sample, one sample of hemlock, and one of spruce needles. However, the counts at 20 to 55 MPN/100 g were low.

Table 1 shows that the samples of bark examined gave a highly variable count. Those from the Seymour watershed, regardless of tree type, gave total coliform counts ranging from $<20$ to $>24{,}000$. The samples from the McMillan Grove Provincial Park and the Douglas fir samples from the logging truck had coliform counts below the level of detection. The sample of Grandfir bark taken from the logging truck, gave a count of 130. The samples taken from the log pond, where the logs were floating in sea water, exposed to municipal sewage discharges gave counts ranging from 140 to $>24{,}000$. Fecal coliform counts were confined solely to those bark samples taken from that log pond.

The completed coliform counts for the fruits and vegetables, expressed as MPN/100 g (wet weight), are listed in Table 2. They show that all but one of the samples examined gave positive total coliform counts exceeding $2 \times 10^6$. To-

TABLE 1. *Coliform counts[a] from forest samples*

| Type of sample | No. of samples | Source[b] | Total completed | Fecal |
|---|---|---|---|---|
| Water | 3 | S | 4–21 | <2–2 |
| Soil | 3 | S | 20–4,300 | <20 |
| Moss | 1 | S | 940 | <20 |
| Fern | 1 | S | 4,300 | 20 |
| Hemlock needles | 2 | S | 790–16,000 | 20 |
| Hemlock needles | 1 | N | <20 | <20 |
| Grandfir needles | 2 | S | 790–1,100 | <20–55 |
| Douglas fir needles | 1 | N | <20 | <20 |
| Cedar needles | 1 | N | 54,000 | <20 |
| Hemlock bark | 1 | N | <20 | <20 |
| Hemlock bark | 4 | LP | 140–>24,000 | <20–>2,000 |
| Grandfir bark | 2 | S | 330–>24,000 | <20 |
| Grandfir bark | 1 | LP | >24,000 | >24,000 |
| Grandfir bark | 1 | LT | 130 | <20 |
| Douglas fir bark | 2 | S | 20–>24,000 | <20 |
| Douglas fir bark | 1 | N | <20 | <20 |
| Douglas fir bark | 4 | M | <20 | <20 |
| Douglas fir bark | 4 | LT | <20 | <20 |
| Cedar bark | 2 | N | <20 | <20 |
| Cedar bark | 1 | S | 1,400 | <20 |

[a] MPN/100 g (wet weight).

[b] Abbreviations: S, Seymour watershed, November, 1970; N, Nanaimo watershed, February, 1971; LP, log pond, May, 1971; LT, logging truck, May, 1971; M, McMillan Grove Provincial Park, May, 1971.

matoes were the exception with a count of 3,300.

The fecal coliform counts listed in Table 2 show that radishes, beets, carrots, and tomatoes gave counts below the minimum number detectable by our procedures, (i.e., less than 200). The leaf lettuce, head lettuce, and whole celery had counts in the vicinity of 400 to 500, whereas the radish tops, beet tops, and carrot tops gave counts between 4,600 and 17,000. The green onions gave a fecal coliform count of $5 \times 10^6$ per 100 g (wet weight).

**Identification of isolates.** A total of 149 isolates from the forest environment were picked from EMB plates; 26 were lactose negative and the remainder were classified by their reactions to the IMViC, $H_2S$, ornithine decarboxylase, and motility tests. The probable classification, source, number, and EC reaction of the remaining isolates are listed in Table 3. Numerically, 71% of the isolates were *Klebsiella* (35% of which were EC positive), 19% were *Enterobacter* (25% EC positive), 8% were *Citrobacter*, and 2 were *Escherichia*, both of which were EC positive.

A total of 152 isolates from the fruit and vegetables were picked from MacConkey plates; six failed to ferment lactose, and the remainder were classified by their reactions to the seven biochemical tests used. The probable classification, source, number, and EC reaction of the isolates are listed in Table 4. Numerically, 64% of the isolates were *Klebsiella*, 14% *Escherichia*, 14% *Enterobacter*, and 8% *Citrobacter*. All the *Enterobacter* and *Citrobacter* were EC negative, whereas 25% of *Klebsiella* and 80% of the *Escherichia* were EC positive.

TABLE 2. *Coliform counts[a] from fresh produce*

| Test samples | Completed total | Fecal |
|---|---|---|
| Radishes | $2.8 \times 10^6$ | <200 |
| Radish tops | $16 \times 10^6$ | 17,000 |
| Leaf lettuce | $>24 \times 10^6$ | 450 |
| Head lettuce | $9.2 \times 10^6$ | 400 |
| Whole celery | $5.4 \times 10^6$ | 400 |
| Beets | $>24 \times 10^6$ | <200 |
| Beet tops | $>24 \times 10^6$ | 7,900 |
| Carrots | $9.2 \times 10^6$ | <200 |
| Carrot tops | $9.2 \times 10^6$ | 4,600 |
| Green onions | $>24 \times 10^6$ | $5.4 \times 10^6$ |
| Tomatoes | 3,300 | <200 |

[a] MPN/100 g (wet weight).

## DISCUSSION

Examination of the data in Table 3 shows that *Klebsiella* and *Enterobacter* were obtained from all sources, and *Citrobacter* from all except soil. *Klebsiella* and *Enterobacter*

TABLE 3. *Characterization of isolates from the forest environment*

| Probable classification | Source | No. of isolates | | Total | Per cent of total isolates |
|---|---|---|---|---|---|
| | | EC negative | EC positive | | |
| *Enterobacter* | Fresh water | 4 | 0 | | |
| | Soil | 2 | 0 | | |
| | Moss, ferns, needles | 1 | 0 | | |
| | Bark | 3 | 0 | | |
| | Bark from log pond | 8 | 6 | | |
| | | (18)[a] | (6) | 24 | 19 |
| *Klebsiella* | Fresh water | 9 | 0 | | |
| | Soil | 3 | 0 | | |
| | Moss, ferns, needles | 16 | 0 | | |
| | Bark | 14 | 1 | | |
| | Bark from log pond | 14 | 30 | | |
| | | (56) | (31) | 87 | 71 |
| *Citrobacter* | Fresh water | 4 | 0 | | |
| | Moss, fern, needles | 4 | 0 | | |
| | Bark | 1 | 0 | | |
| | Bark from log pond | 1 | 0 | | |
| | | (10) | (0) | 10 | 8 |
| *Escherichia* | Fresh water | 0 | 2 | | |
| | | (0) | (2) | 2 | 2 |
| | Total | 84 | 39 | 123 | |

[a] Numbers in parentheses are subtotals.

were obtained from all types of bark which gave positive counts, and so no species differentiation is listed in the table. The two *Escherichia* isolates were both obtained from the stream sample taken in the Seymour watershed. No *Escherichia* isolates were found on the bark of logs taken from the log pond, which receives settled but unchlorinated sewage.

Stewart et al. (21) found that a municipal watershed, closed to public entry since 1917, yielded water with a coliform count four to six times that of an adjacent watershed open to recreational activity. It was suggested that the closed watershed had a large population of wild animals that contributed substantially to the results obtained in their studies. Unfortunately, the authors did not classify their coliform isolates by genus.

Examination of the data in Table 4 does not demonstrate any specific relationship between a particular genus and a type of produce. *Klebsiella* was isolated from all sources, and *Enterobacter* was isolated from all but the head lettuce and the carrots. There was no *Citrobacter* present on the celery, the carrots, carrot tops, or on the tomatoes. *Escherichia* was present on the least number of samples. This genus was absent from leaf lettuce, celery, and tomatoes and the underground portions of radishes, beets and carrots, but was present on the tops of radishes, beets, and carrots. Interestingly enough, the underground portions of the vegetables were also free of any EC-positive isolates. The extremely high EC-positive count associated with green onions was primarily due to the presence of *K. pneumoniae*. Shooter et al. (20) found *Klebsiella* species on 21 of the 121 salads they examined in their study and *E. coli*

TABLE 4. *Characterization of isolates from fresh produce*

| Probable classification | Source | No. of isolates | | Total | Per cent of total isolates |
|---|---|---|---|---|---|
| | | EC negative | EC positive | | |
| *Enterobacter* | Radishes | 2 | 0 | | |
| | Radish tops | 2 | 0 | | |
| | Leaf lettuce | 2 | 0 | | |
| | Whole celery | 3 | 0 | | |
| | Beets | 2 | 0 | | |
| | Beet tops | 1 | 0 | | |
| | Carrot tops | 6 | 0 | | |
| | Green onions | 1 | 0 | | |
| | Tomatoes | 1 | 0 | | |
| | | (20)[a] | (0) | 20 | 14 |
| *Klebsiella* | Radishes | 4 | 0 | | |
| | Radish tops | 5 | 4 | | |
| | Leaf lettuce | 9 | 0 | | |
| | Head lettuce | 7 | 0 | | |
| | Whole celery | 3 | 2 | | |
| | Beets | 6 | 0 | | |
| | Beet tops | 7 | 4 | | |
| | Carrots | 11 | 0 | | |
| | Carrot tops | 7 | 0 | | |
| | Green onions | 7 | 13 | | |
| | Tomatoes | 5 | 0 | | |
| | | (71) | (23) | 94 | 64 |
| *Citrobacter* | Radishes | 2 | 0 | | |
| | Radish tops | 1 | 0 | | |
| | Leaf lettuce | 1 | 0 | | |
| | Head lettuce | 1 | 0 | | |
| | Beets | 2 | 0 | | |
| | Beet tops | 2 | 0 | | |
| | Green onions | 2 | 0 | | |
| | | (11) | (0) | 11 | 8 |
| *Escherichia* | Radish tops | 0 | 6 | | |
| | Head lettuce | 2 | 0 | | |
| | Beet tops | 2 | 4 | | |
| | Carrot tops | 0 | 6 | | |
| | Green onions | 0 | 1 | | |
| | | (4) | (17) | 21 | 14 |
| | Total | 106 | 40 | 146 | |

[a] Numbers in parentheses are subtotals.

on 26 of 158. However, the counts appeared lower than those reported here because the salad greens had been washed.

The data presented here indicate that, when seven biochemical tests are applied to lactose-fermenting *Enterobacteriaceae*, in conjunction with the classification system of Ewing and Edwards (9), approximately 70% of the isolates from the forest and 65% from fresh produce are classified as *Klebsiella*, and predominantly as *K. pneumoniae* (Table 5), an organism which is currently considered to be pathogenic. Before the *Enterobacteriaceae* was reclassified, these organisms probably would have been known as *A. aerogenes*, a name with nonpathogenic connotations. Cowan et al. (5) recognized this similarity in biotypes and retained the name *K. aerogenes* for those biotypes which were IMViC − − + +, but HOMoC − − − +, a nomenclature which was supported by Bascomb et al. (2) in their numerical classification of the tribe *Klebsielleae*. Both these authors classify *K. pneumoniae* as being IMViC − + − +, and only 10 of the 181 *Klebsiella* isolated in this study fit that definition. Such organisms would tentatively be classified as *K. ozaenae* according to Fife et al. (10).

In their study of the tribe *Klebsielleae*, Fife et al. (10) recognized that not all biotypes fit the classic definition, and they listed percentage values to indicate frequency. In our study no one combination of reactions for the seven tests used described the majority of the *Enterobacter* biotypes. The classic IMViC − − + + reaction described 30 out of the 43 isolates, whereas the HOMoC classification of − + + + suggested by Closs and Digranes (4) identified 32 of the 43 isolates. Each classification included 50% of the EC-positive isolates. With *Klebsiella*, 109 of the 181 isolates could be classified by the classic IMViC − − + + reactions (Table 5). All but 6 of the isolates were HOMoC − − − +.

All non-*Klebsiella* isolates which were MViC + − + and did not liquefy gelatin were classed as *Citrobacter*. Eighteen of the 21 isolated were $H_2S$ negative. The 21 *Escherichia* isolates were all IMViC + + − −, and 19 of the 21 were HOMoC − + + −.

The results of this study show that a majority of the coliform group isolates taken from forest-related samples and from fresh produce were *Klebsiella* and predominantly *K. pneumoniae* according to the classification scheme of Ewing and Edwards (9). Previous classification schemes would probably have assigned these organisms to the genus *Aerobacter*. The Ewing and Edwards classification system evolved through the examination of large numbers of *Enterobacteriaceae* which were primarily clinical isolates. It remains to be seen if the *Klebsiella* isolated from the nonmedical environment are of equal pathogenicity to those isolated from clinical sources. [Some data indicate that the mouse pathogenicity of environmental and hospital isolates are indistinguishable, whereas the latter show patterns of multiple antibiotic resistance (J. M. Matsen, *personal communication*).] Serological typing and antibiotic susceptibility testing of our isolates are planned.


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TABLE 5. *Frequency of combination of various characteristics in Klebsiella isolates*

| Reaction to tests performed | | | | | | | | Probable classification | No. of isolates | | Total | Per cent of all *Klebsiella* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | M | Vi | C[a] | H | O | Mo | C[b] | | Forest environment | Produce | | |
| − | − | + | + | − | − | − | + | *K. pneumoniae* | 51(22 EC+) | 58(22 EC+) | 109 | 60.2 |
| − | − | + | − | − | − | − | − | *K. pneumoniae* | 1 | 0 | 1 | 0.6 |
| − | − | − | + | − | − | − | + | *K. pneumoniae* | 0 | 1(EC+) | 1 | 0.6 |
| − | + | + | + | − | − | − | + | *K. pneumoniae* | 19(3 EC+) | 8 | 27 | 14.9 |
| + | − | + | + | − | − | − | + | *K. pneumoniae* | 0 | 11 | 11 | 6.0 |
| + | + | + | + | − | − | − | + | *K. pneumoniae* | 2 | 14 | 16 | 8.8 |
| + | + | − | + | − | − | − | + | *K. pneumoniae* | 0 | 1 | 1 | 0.6 |
| − | + | + | − | − | − | − | − | *K. pneumoniae* | 4 | 0 | 4 | 2.2 |
| − | + | − | + | − | − | − | + | *K. ozaenae* | 9(6 EC+) | 1 | 10 | 5.5 |
| − | + | − | − | − | − | − | − | *K. rhino-scleromatis* or *K. ozaenae* | 1 | 0 | 1 | 0.6 |

[a] Indole, methyl red, Voges-Proskauer, citrate.

[b] $H_2S$, ornithine decarboxylase, motility, citrate.

## LITERATURE CITED


1. American Public Health Association. 1971. Standard methods for the examination of water and waste water, 13th ed. American Public Health Association, New York.
2. Bascomb, S., S. P. LaPage, W. R. Willcox, and M. A. Curtis. 1971. Numerical classification of the tribe *Klebsielleae*. J. Gen. Microbiol. **66**:279-295.
3. Blosser, R. O. 1971. Experience with indicator organism tests in determining the bacteriological quality of pulp and paper mill effluents and their receiving waters. National Council of the Paper Industry for Air and Stream Improvement. Tech. Bull. No. 244. 56 p.
4. Closs, O., and A. Digranes. 1971. Rapid identification of prompt lactose-fermenting genera within the family *Enterobacteriaceae*. Acta Pathol. Microbiol. Scan. Sect. B **79**:673-678.
5. Cowan, S. T., K. J. Steel, C. Shaw, and J. P. Duguid. 1960. A classification of the *Klebsiella* group. J. Gen. Microbiol. **23**:601-612.
6. Dutka, B. J., J. B. Bell, P. Collins, and J. Popplow. 1969. A bacteriological study of the Rainy River, conducted for the Advisory Board on Water Pollution, Rainy River, and Lake of the Woods, International Joint Commission. Department of National Health and Welfare (Canada). Div. Pub. Health Eng. Manuscript Rep. KR-69-1. 42p.
7. Eickhoff, T. C. 1972. *Klebsiella pneumoniae* infection: a review with reference to the water-borne epidemiologic significance of *K. pneumoniae* presence in the natural environment. National Council of the Paper Industry for Air and Stream Improvement. Tech. Bull. 254. 24 p.
8. Edwards, P. R. 1929. Relationships of the encapsulated bacilli with special reference to *Bact. aerogenes*. J. Bacteriol. **17**:339-353.
9. Ewing, W. H., and P. R. Edwards. 1960. The principal divisions and groups of *Enterobacteriaceae* and their differentiation. Int. Bull. Bacteriol. Nomencl. Taxon. **10**:1-12.
10. Fife, N. A., W. H. Ewing, and B. R. Davis, 1965. The biochemical reactions of the tribe *Klebsielleae*. Communicable Disease Center, Atlanta, Ga.
11. Frazer, M. G., W. B. Reid, and J. F. Malcolm. 1956. The occurrance of coli-aerogenes organisms on plants. J. Appl. Bacteriol. **19**:301-309.
12. Geldreich, E. E., and R. H. Bordner. 1971. Fecal contamination of fruits and vegetables during cultivation and processing for market. A review. J. Milk Food Technol. **34**:184-195.
13. Geldreich, E. E., B. A. Kenner, and P. W. Kabler. 1964. Occurrance of coliforms, fecal coliforms, and streptococci on vegetation and insects. Appl. Microbiol. **12**:63-69.
14. Martin, R. B., and J. G. Dobson. 1945. Identification and characteristics of typical slime forming organisms. Pap. Trade J. **121(15)**:39-46.
15. Martin, W. J., P. K. W. Yu, and J. A. Washington. 1971. Epidemiologic significance of *Klebsiella pneumoniae* —three month study. Mayo Clin. Proc. **46**:785-793.
16. Nunez, W. J., and A. R. Colmer. 1968. Differentiation of *Aerobacter-Klebsiella* isolated from sugar cane. Appl. Microbiol. **16**:1875-1878.
17. Papavassiliou, J., S. Tzannetis, H. Leka, and G. Michopoulos. 1967. Coli-aerogenes bacteria on plants. J. Appl. Bacteriol. **30**:219-223.
18. Reid, J. E., A. D. Tennant, and L. J. Rockwell. 1961. A bacteriological study of the Rainy River, conducted for the advisory Board on Water Pollution, Rainy River and Lake of the Woods, International Joint Commission. Department of National Health and Welfare (Canada). Manuscript Rep. Lab. Hyg. 61-1. 51 p.
19. Reid, J. E., A. D. Tennant, and L. J. Rockwell. 1963. A bacteriological study of the Saint Croix River, International Joint Commission. Department of National Health and Welfare (Canada). Manuscript Rep. Lab. Hyg. 63-1. 34 p.
20. Shooter, R. A., M. C. Faiers, E. M. Cooke, A. L. Breaden and S. M. O'Farrell. 1971. Isolation of *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* from food in hospitals, canteens and schools. Lancet. No. 7721, p. 390-393.
21. Stuart, D. G., J. K. Bissonnette, T. D. Goodrich, and W. G. Walter. 1971. Effects of multiple use on water quality of high-mountain watersheds: bacteriological investigations of mountain streams. Appl. Microbiol. **22**:1048-1054.
22. Tennant, A. D., J. E. Reid, and L. J. Rockwell. 1960. A bacteriological study of the Rainy River, conducted for the Advisory Board on Water Pollution, Rainy River, and Lake of the Woods, International Joint Commission. Department of National Health and Welfare (Canada). Manuscript Rep. Lab. Hygiene 60-1, 52 p.
23. Thomas, S. B., and J. McQuillin. 1952. Coli-aerogenes bacteria isolated from grass. Proc. Soc. Appl. Bacteriol. **15**:41-52.