A STUDY
OF
BACTERIA OF THE ESCHERICHIA-AEROBACTER GROUP
RESPONSIBLE FOR
AN ALLEGED FEED FLAVOUR AND STABLE ODOUR IN MILK

by

Vivienne G. Hudson, B. A. and C. Duncan MacKenzie, B. S. A.

A Thesis Submitted for the Degree of

MASTER OF ARTS

in the Department

of

BACTERIOLOGY

and for the Degree of

MASTER OF SCIENCE IN AGRICULTURE

in the Departments

of

ANIMAL HUSBANDRY and DAIRYING

Accepted

With One Table

The University of British Columbia

April 1932

A STUDY
OF
BACTERIA OF THE ESCHERICHIA - AEROBACTER GROUP
RESPONSIBLE FOR
AN ALLEGED FEED FLAVOUR AND STABLE ODOUR IN MILK

The work reported upon by Sadler, Irwin and Golding [1] and by Sadler and Irwin [2] suggested the critical necessity of a comprehensive investigation being made into the relation of bacteria to the incidence of a so-called feed flavour and stable odour in milk.

Cast in a somewhat popular mould, describing in detail how the enquiry came to be engaged in, the former paper [1] has shown that an alleged feed flavour and stable odour common to milk, is to be attributed to specific strains of aerobic gas-producing bacteria; and that this so-called feed flavour can be detected by the association therewith of a penetrating nauseating and characteristic odour. The micro-organism found to be responsible for the defect was isolated from corn silage, but no evidence is to be adduced that this organism is present in all corn silage as such. Sadler and Irwin [2] describe the cultural characteristics of the organism and classify it as an atypical strain of

Aerobacter oxytocum [2] (Migula), Bergey et al [2] [3].

On entering upon the more comprehensive study with which the present paper is concerned, it appeared to be essential to examine representative milk supplies, the various feeds that go to make up the rations of milk-producing cows, and also such other sources or materials as might be a possible habitat for organisms capable of producing this characteristic feed flavour and stable odour in milk. Moreover, the possibility of implements and equipment to be found on farm premises being carriers of the feed flavour producing bacteria had to be borne in mind. The typical organism already studied and reported upon [2] having proved to be a strain of Aerobacter oxytocum, it was decided to follow such procedures and use such methods and media as would make possible the isolation of bacteria of the Escherichia-Aerobacter group in particular.

It seemed reasonable to conjecture that providing we could secure data on the relation of bacteria to feed flavours and stable odours in milk, some contribution to knowledge would be assured and those things that constitute good management on milk-producing farms might be the more clearly defined.

METHODS AND PROCEDURES

It became necessary to adopt, adapt, apply and employ methods and procedures suitable for the securing of samples of the material to be examined and likewise suitable for bacteriological examinations of such material.

The Taking of Samples

In the collecting of all samples the usual aseptic precautions were observed throughout.

MILK: Using sterile pipettes and bottles, samples of milk--morning's, evening's and mixed--were collected after the arrival of the milk supply at the dairy or at the receiving station respectively.

FEEDS: With sterile forceps and spoons, samples of feeds were collected in sterile containers.

FARM PREMISES: In order to examine farm premises, equipment and implements, swabs--such as those in use in hospital practice--were employed, the swabs having been sterilized previously in the usual manner.

Examination of Material

MILK: Inoculations of 1 c.c., 1/10 c.c., and 1/100 c.c. of each sample of milk to be examined, were made in duplicate into flasks of sterile milk.# One series was incubated at 37°C. and the duplicate at 30°C. After 24 hours' incubation the flasks of inoculated milk were examined for the presence of the characteristic stable odour. It was found that if the odour developed at all it was as pronounced at 30°C. as at 37°C., and in many cases even more pronounced at the lower temperature. Thereafter, the routine procedure was to incubate at 30°C. for 24 hours when seeking to detect the typical odour in milk. From all flasks in which the feed flavour odour could be defined, or in which the production of gas and frothing were evident, plates were made in varying dilutions on neutral red bile salt agar.# The plates were incubated for 48 hours at 30°C. and were then examined for the colonies characteristic of organisms of the Escherichia-Aerobacter group. Tubes of litmus milk # were inoculated from common colonies and after incubation were examined for gas production or frothing, and for acidity. From all tubes in which gas production was evident, 1 c.c. of the milk was inoculated into 250 c.c. quantities of sterile milk in flasks,# and these were later examined for the characteristic feed flavour odour and for frothing. When the

For details as to media see page 6.

defect could be detected, the culture was transferred to agar # and set aside to await further study.

FEEDS: Small quantities of the feed samples secured were inoculated into flasks of sterile milk for examination. If the smell of the particular feed as such prevailed after incubation transfers (1 c.c.) into flasks of sterile milk were made from the milk containing the original gross inoculation. On the detecting of the characteristic odour neutral red bile salt agar plates were made, the subsequent procedures being as already described.

SWABS: The swabs were transferred from their test tube containers into tubes of litmus milk. From all tubes that showed production of gas, 1 c.c. inoculations were made into flasks of milk. The same procedure described above was followed for isolating the organisms.

Employing these methods of examination, strains of aerobic gas-producing bacteria, each of which produced to a greater or lesser degree the characteristic feed flavour and stable odour in milk were isolated from: milk, the tongues of dairy cows, baled alfalfa hay in the feed room, scrapings from dairy stable mangers, alfalfa from a manger, grain mixtures from a feed truck, feed room, and granary bins, pieces of cut mangles from the slicer, soaked beet

pulp from the feed room, water from a drinking bowl attached to stanchions, a hay truck in dairy barn, a beet pulp shovel, mangel forks, mangel cutters in feed rooms, milk sheet blotters in milk room attached to dairy barn, the floor in a feed room, the floor where grain is mixed, straw bedding in dairy stalls, the floors behind stalls, a milk stool, a grooming brush in dairy stable, dairy barn gutters, the wall in a dairy barn, the rafters in a barn, stanchions, a ditch, mud from a paddock, and stable manure. These described sources and the characteristics of the bacteria isolated therefrom are set forth in Table I.

THE CULTURAL STUDY

Media Employed

Agar, gelatine and the various differential media used in the study were prepared from the dessicated media of the Digestive Ferments Company of Detroit, Michigan, and were sterilized for 20 minutes at 14 pounds pressure.

Glucose Agar:

Difco nutrient agar in which 1% glucose was dissolved before sterilizing.

Milk:

For the detecting of smell and odour, skim milk in flasks (250 c.c. quantity) steamed for 45 minutes on one, two, or three successive days.

Litmus Milk:

Approximately 8 c.c. skim milk--with added azolitmin solution--filled into test tubes and sterilized for 20 minutes at 12 pounds pressure.

Carbohydrates for Fermentation Studies:

With the exception of glycerine and adonite, all carbohydrates employed were obtained from the Digestive Ferments Company.

The method of preparing the sugar media and the procedure followed in the fermentation determinations are here excerpted from the former paper (2):

"For the determination of the acid and gas formed from the various substances used, the shake agar method was employed. With the exception of adonite on account of its cost, and of aesculite, because of the difficulty of using a greater concentration, which were added to the extent of 0.5%, the carbohydrates were added to nutrient agar to the extent of 2%. Brom-cresol-purple was used as an indicator. Portions of about 8 cc. of the various sugar media were placed in tubes 6 in. long and 0.6 in. in diameter, and these placed in an autoclave for 20 min. under 13 lb. pressure. A 17-hr. old nutrient broth culture grown at 37°C., was used for inoculating. In order to get results as closely comparable as possible, a similar amount of the broth--the quantity held by a loop 2 mm. in diameter--was added to each melted sugar agar. Each agar tube was immediately rotated in such a manner that the medium was not distributed around its walls, and it was placed in cold water. After setting, the inoculated tubes were placed in a water-bath so as to reach as rapidly as possible the approximate temperature of incubation; they were then transferred to the incubator kept at 37°C. The tubes were examined after an incubation of four hours and at regular intervals to 10 and 12 hr. incubation. All inoculated media were again examined after 24 hr. Each tube being removed from the incubator to be examined was held in a water-bath at a suitable temperature."

The following carbohydrates and higher alcohols were used: dextrose, lactose, sucrose, dulcite, salicin, glycerine, mannite, sorbite, adonite, xylose, arabinose, rhamnose, laevulose, mannose, galactose, maltose, raffinose, dextrin, inulin, soluble starch, aesculin.

Bacteria Submitted to Study

One hundred and four organisms that had definitely produced the characteristic feed flavour and stable odour in milk were repurified and retained for study. Thereafter, the procedures adopted and the methods employed followed closely those described in the paper already cited (2).

The results of the detailed cultural study are summarized in Table I.

AN ANALYSIS OF THE DATA IN TABLE I

Of the one hundred and four organisms that produce the typical feed flavour and odour in milk, one strain culture No. 212, a short Gram negative rod fails to produce gas in any of the carbohydrates employed. Another strain No. 20, appearing under the microscope as long slender rods, produces acid and gas from lactose but fails to ferment dextrose to gas. Throughout the present discussion, no further mention will be made of cultures No. 20 and 212.

One hundred and two strains that ferment lactose to acid and gas--the presumptive test for bacteria of the Escherichia-Aerobacter (coli-aerogenes) group--appear under the microscope as very short Gram negative rods.

The Bacteria That Are Positive to the Voges-Proskauer Test

Fifty-eight of the lactose fermenting strains are positive to the Voges-Proskauer test # and of these, thirty-five are alkaline to Methyl Red. Table I. Thus the correlation of the results of the Voges-Proskauer and Methyl Red reactions is established for thirty-five of the fifty-eight organisms definitely positive to the Voges-Proskauer test. Of the remaining twenty-three cultures that are positive to the Voges-Proskauer test, a correlation is not to be found. Four strains, No. 206, 137, 139, 198, are

It has been observed by Sadler and Irwin (2) that their Aerobacter oxytocum (atypical) strain gave a positive reaction to the Voges-Proskauer test after 24 hours incubation, and failed to do so after 96 hours incubation. In the present study fifty-three of the fifty-eight organisms gave a positive Voges-Proskauer test after 24 hours incubation at 30°C. and 37°C. respectively. Five strains, No. 206, 251, 32, 52 and 116 were positive to the test in 48 hours. Judged by the Voges-Proskauer test, no acetyl-methyl-carbinol could be detected after 96 hours incubation in fifteen of these strains--cultures No. 24, 249, 261, 275, 282, 109, 110, 112, 113, 114, 115, 122, 34, 67, 116.

In view of the observations of Durham (4) and Levine et al (5) we think it of interest to report that even after an incubation period of 8 hours we got a positive Voges-Proskauer reaction with cultures No. 24, 249, 261, 265, 275, 276, 277, 283, 49, 198, 109, 110, 112, 113, 114, 115, 122, 66, 68, 34, 67, 119, 121, 166, 167, 168, 169, 170, 188, 229, 47, 197, 209a, 209b, 120.

definitely acid to Methyl Red, while nineteen are variable to the Methyl Red reaction after periods of 48 and 72 hours incubation of 30°C. and 37°C. respectively. As precautions were taken to insure purity of the cultures studied, we can offer no explanation of this variation to the Methyl Red reaction of strains that unmistakeably produce acetyl-methyl-carbinol. The cultures that produce acetyl-methyl-carbinol and are variable to the Methyl Red reaction are No. 161, 162, 163, 164, 51, 66, 127, 213, 251, 53, 67, 117, 118, 119, 121, 229, 52, 116, 120. Discrepancies such as these to which we have drawn attention, have already been observed by Johnson and Levine (6).

Proceeding with the analysis of the characteristics of the organisms that produce acetyl-methyl-carbinol, it is seen from Table I that thirty-two strains fail to liquefy gelatine and twenty-six are gelatine liquefiers: fifteen are motile and forty-three are non-motile: seven strains only form indol: all of the fifty-eight reduce nitrates to nitrites: and the production of gas in litmus milk in all cultures was pronounced.

For the fermentation studies, twenty-one carbohydrates were employed. As can be seen from Table I, an attempt has been made to give a comparative idea of the extent to which the individual organisms attack the carbohydrates: furthermore, an indication is to be seen of the preference shown by particular organisms for specific sugars--

observations being made after incubation for 4, 8, 10, 24, 48 and 72 hours respectively. In all cases, when the organisms produced acid and gas, little further development in gas production took place after the 24 hour incubation period; whilst the reaction of all cultures to the carbohydrates used by Bergey et al [3] in the differentiation of species within the genus Aerobacter was specific at this time. Continuing the incubation period for a further 24 hours served to accentuate the differentiating value of certain of the sugars and higher alcohols. For instance, the gas production from xylose by cultures No. 161, 162, 163, 164, 127, 213, 121 and 32 was more pronounced: likewise, more gas formation was evident in the maltose agar tubes inoculated with cultures No. 49, 137, 139, 127, 34, 67 and 72, and the attack on the glucoside aesculin was more definite by cultures No. 127, 117, 118, 119, 121, 229, 72 and 19. The production of acid and gas from arabinose by culture No. 120 and from dextrin by cultures No. 24, 249, and 275 was accentuated by continuing the incubation for 72 hours.

The Bacteria That Are Negative to the Voges-Proskauer Test

Of the lactose fermenting organisms there are forty-four strains that are acid to Methyl Red and do not produce acetyl-methyl-carbinol. All fail to liquefy gelatine: thirty are motile and fourteen are non motile: forty produce

indol and all reduce nitrates to nitrites. Except cultures No. 144, 123, 146, and 99, all produced gas in litmus milk. Usually the gas production was not as pronounced as it was in the case of the organisms positive to the Voges-Proskauer test.

In the fermentation studies employing the twenty-one carbohydrates, observations were made in a manner identical with that described above. Except in the production of gas from dulcite and salicin by culture No. 40, from dulcite by culture No. 44, and from salicin by cultures No. 66 and 58, the presence of acid and gas when produced at all, was definite after 24 hours incubation; and the differentiation of species within the genus Escherichia, after Bergey et al (3), was specific at this time. The 48 hours incubation period served to accentuate the action of certain of the cultures on specific carbohydrates: for instance, the gas production from dulcite by cultures No. 57, 63, 146 and 222, from salicin by cultures No. 45, 46, 54, 123, 4 and 85, and from maltose by cultures No. 50, 21, 22 and 222 was more pronounced. The production of acid and gas from dulcite by culture No. 263, from salicin by culture No. 64, from glycerine by cultures No. 240 and 269 and from aesculin by cultures No. 45, 54, 43, 175, 85, 146, and 41, was accentuated by continuing the incubation period for 72 hours.

THE CLASSIFICATION OF THE BACTERIA

In attempting the classification of the bacteria, we have considered the characteristics as summarized in Table I, and throughout, have kept before us, the qualifications to which we have drawn attention in the foregoing critical analysis of the reactions of the bacteria. In defining the genus and the species of each of the organisms, we have followed in the main, Bergey et al [3].

In the analysis of the data on Table I, we drew attention to two cultures, No. 212 and 20 and for the reasons given there, we refrained from including the two organisms in the discussion. In the absence of other strains showing identical characteristics, we propose to offer no suggestion as to the classification of these two organisms. Consequently, in the classification of the bacteria studied, we are here concerned with one hundred and two organisms.

The motile or non-motile rods that are Gram negative, that form gas from dextrose and lactose, and that produce acetyl-methyl-carbinol, are placed within the genus Aerobacter, Beijerinck, Bergey et al [3].

On the sum of the characteristics determined, Table I, cultures No. 161, 162, 163, 164, 165 and 206 are classified as <u>Aerobacter oxytocum</u> (Migula) Bergey et al [3],

even though culture No. 206 fails to produce indol, and forms no gas in inulin. Cultures No. 24, 249, 261, 265, and 275 are identical, the one with the other, and with the atypical strain of Aerobacter oxytocum [3] reported upon by Sadler and Irwin [2]. Cultures No. 276, 277, 282, and 283 are also classified as atypical strains of Aerobacter oxytocum [3] [2].

Each of the cultures No. 49, 51, 137, 139 and 198 is classified as Aerobacter aerogenes, (Kruse) Beijerinck, [3] and cultures No. 109, 110, 112, 113, 114, 115, and 122 which fail to attack adonite, are to be considered as being atypical strains of the same species.

If we consider only the characteristics used by Bergey et al [3] for the major differentiation of species within the genus Aerobacter, cultures No. 66 and 68 appear to be related to Aerobacter aerogenes [3]; culture No. 127 in some measure to Aerobacter levans (Wolffin) Bergey et al [3], and cultures No. 213 and 251 to Aerobacter aerogenes [3]. In the case of each of these strains, however, very specific variations from the respective types are to be seen--Bergey et al [3] and Table I of this paper: hence, considering the characteristics as a whole, we place these cultures within the genus Aerobacter but refrain from attempting a more precise classification.

As can be seen from Table I, many of our strains are motile or non-motile Gram negative rods, produce acetyl-methyl-carbinol, ferment sucrose to acid and gas, and liquefy gelatine rapidly or slowly. According to Bergey et al [3] and in the light of the data on Table I certain of these cultures, to wit, No. 72, 19, 47, 71, 197, 209a, 209b, 32, 52, 116, 120 and 220 might be considered as strains of Aerobacter bombycis, Bergey et al [3]. Yet, on the other hand, based on the sum of the characteristics, these cultures are to be recognized as variants of Aerobacter cloacae, after Jordan [7], [3]: and the failure of the strains to agree the one with the other in the ability to attack certain of the carbohydrates, notwithstanding, we suggest that cultures No. 72, 19, 47, 71, 197, 209a, 209b, 32, 52, 116, 120 and 220 be classified as variants of Aerobacter cloacae, (Jordan) [7] Bergey et al [3]. Cultures No. 34, 53, 67, 117, 118, 119, 121, 166, 167, 168, 169, 170, 188, and 229 are the non-motile strains of the group of organisms whose main characteristics are summarized above. These strains fail to agree the one with the other in the ability to ferment certain of the carbohydrates employed in the study, and even though the original strains of Jordan all proved to be motile [7] we feel justified, considering the sum of the characteristics, in placing, tentatively, cultures No. 34, 53, 67, 117, 118, 119, 121, 166, 167, 168, 169, 170, 188, and 229 as

non-motile variants of Aerobacter cloacae (Jordan) [7], Bergey et al [3].

The Gram negative motile or non-motile rods, that produce gas from dextrose and lactose and do not produce acetyl-methyl-carbinol from dextrose are placed within the genus Escherichia, Castellani and Chambers, Bergey et al [3].

Of cultures No. 45, 46, 54, 56, 58, 64, 144 and 240, No. 54, 144, and 240 fail to attack raffinose, none produce gas from dextrin and as can be seen from Table I, identical reactions in all the carbohydrates employed in the study cannot be observed. Yet, in considering the characteristics as a whole, Table I, each of these cultures is to be classified as Escherichia coli (Escherich) Castellani and Chambers, Bergey et al [3].

On the major characteristics, culture No. 263 is classified as Escherichia enterica (Castellani and Chambers) Weldin, Bergey et al [3]: and, cultures No. 12 and 123, which are identical with cultures No. 263 except that they are very active in adonite, are classified as adonite fermenting strains of Escherichia enterica [3].

Cultures No. 40, 43, 44, 57, 181, 192, 194, 233, 236, 245, 175, 267 and 269 form indol, and certain of them, Table I, fail to attack dulcite. Even so, on the characteristics as a whole, these organisms must be looked upon as strains of Escherichia formica [3] and we place

them as indol producing variants of Escherichia formica (Omelianski) Bergey et al [3].

In so far as the literature permits of comparison, the cultural characteristics of our strains No. 61 and 63, we suggest that each be classified as Escherichia vesiculiformans (Henrici) Bergey et al [3].

Cultures No. 4, 16, 17, 85, and 145 vary in some measure from Escherichia communior [3] in their action on some of the higher sugars and they appear to be specific in that they are weak in the fermentation of sucrose and salicin, Table I. Whether or not this latter characteristic is a distinct variation in "degree" from Escherichia communior [3] cannot be said: not overlooking this qualification we classify each of cultures No. 4, 16, 17, 85, and 145 as Escherichia communior (Durham) Bergey et al [3]. Cultures No. 130, 146, and 219 fail to produce gas from salicin and consequently in one of the principal sugars used in differentiation [3] a distinct variation from the type Escherichia communior [3] is shown. Even so, we consider we shall be subscribing to the principles that should guide in classifying if on the sum of the characteristics, we define cultures No. 130, 146 and 219 as non-salicin fermenting strains of Escherichia communior (Durham) Bergey et al [3].

Of the non-motile forms included in the genus Escherichia, cultures No. 50, 21, and 22 ferment adonite to

acid and gas and produce in Simmon's Citrate Agar, the blue color which is significant of the genus Aerobacter. Culture No. 50 forms indol whilst cultures No. 21 and 22 do not. These and less critical variations notwithstanding, the strains without doubt are closely related to the typical Escherichia neapolitana, Table I, Bergey et al [3]: but in view of the variations we classify cultures No. 50, 21 and 22 as atypical strains of Escherichia neapolitana (Emmerich) Bergey et al [3]. As non salicin fermenting strains of Escherichia neapolitana Bergey et al [3], we place cultures No. 28, 41, 39, 99, 222, on the sum of their characteristics.

Cultures No. 13 and 255, in view of the characteristics shown in Table I, are difficult to define. Consequently, we leave them within the genus Escherichia but refrain from suggesting a more precise and detailed classification.

THE PRODUCTION BY THE ORGANISMS STUDIED OF THE CHARACTERISTIC FEED FLAVOUR AND STABLE ODOUR IN MILK

These observations were made on flasks of milk # inoculated with the respective bacteria. Rather than attempt to give a description of the characteristic feed flavour and

See page 6.

stable odour, we excerpt direct from the paper of Sadler, Irwin and Golding [1]:

> "The flavor produced in steamed or sterilized milk....is very difficult to define. The taste is disagreeable and definitely unclean. There is an astringent effect, and a slight tingling sensation on the tongue and on the palate. It is not necessary, however, to taste the milk to be sure that the defect is there. The odor is an unfailing guide. On taking the cotton wool plug out of the neck of the flask the characteristic odor can be noted at once. If, however, the flask be shaken vigorously for a moment, the odor is accentuated, is more pungent and penetrating. The shaking produces a very heavy foam, and the milk culture fizzes almost as a syphon of soda water. When the odor characteristic of a defect is present, one is reminded of a combination of smells--a combination of the foeted close air that may be encountered on going into a badly ventilated heavily populated cowshed early of a morning; the mixture of smells that the remains of various feeds given to the cattle the night before can produce; and the stale, slightly sour penetrating smell that memory associates with the clothes of the old-time cowman, to whom the actual milking of the cows was not always the most important part of an arduous day's work. Withal, in the odor is a pungent, nauseating quality not easily to be mistaken....."

Except for cultures No. 53, 72, 47, and 71, all of the organisms we have placed within the genus Aerobacter, produced, when first isolated, a feed flavour characteristically identical with the description already given [1]. In the case of cultures No. 53, 72, 47 and 71, the flavour and odour were definite but not nearly so intense. When first isolated, twenty-three of the forty-four organisms, that we have classified as within the genus Escherichia,

produced the odour and flavour described as characteristic [1]. While still quite definite, the intensity of the flavour and odour was not quite so pronounced in milk inoculated respectively with the other twenty-one strains placed within the genus Escherichia--cultures No. 45, 46, 54, 56, 58, 64, 144, 40, 43, 44, 57, 269, 61, 63, 85, 130, 146, 219, 41, 39 and 99.

After the organisms had been in artificial media for some six months, it was found that except cultures No. 66 and 68, all those of the genus Aerobacter produced a truly characteristic feed flavour and stable odour to a pronounced degree. Not only is it of particular interest to find that after this period in pure culture, the ability to produce the characteristic feed flavour and stable odour was thus far a constant characteristic of the majority of these organisms, but it is to be observed that the four strains which, on isolation had produced the defect in some measure, now gave the flavour and odour of an intensity comparable with the flavour and odour originally described [1].

After a similar period in artificial media, thirty-three of the organisms of the genus Escherichia gave a pronounced and characteristic feed flavour and stable odour--cultures No. 45, 46, 54, 56, 144, 240, 12, 263, 40, 57, 181, 192, 194, 233, 236, 245, 175, 267, 269, 4, 16, 17, 85, 145, 130, 50, 21, 22, 28, 41, 39, 99 and 255. The remaining eleven

organisms placed in this genus produced the defect in lesser measure. It will not be overlooked that, when making observations on a defect such as this with which we are concerned, it is extremely difficult to define intensity of smell in a manner more precise than say slight, marked or pronounced. Even so, quite definitely our study permits us to report that certain cultures were, over a period of time, consistent in that they produced the characteristic feed flavour and stable odour to a greater or lesser degree respectively throughout.

On the whole, the bacteria classified as within the genus Aerobacter, produced a more pronounced feed flavour and stable odour than did the organisms finding themselves within the genus Escherichia.

POSSIBLE CHANNELS OF INFECTION

As a ready means whereby one may see something of the relationship of known strains to the sources from which respectively they have been isolated, a series of pictorial designs has been prepared. These pictorial designs give one an idea as to possible channels through which the infection of milk by organisms responsible for the alleged feed flavour and stable odour may proceed. As an example three such pictures or diagrams are given here--Diagrams A, B, and C.

Considering Diagram A, it will be seen that the atypical Aerobacter oxytocum (2) (3) has been recovered from corn silage, (1) (2) manger scrapings, milk, the tongue of a cow, alfalfa from a manger, straw used for bedding, and from grain mixtures. According to Diagram B, Aerobacter aerogenes has been recovered from a mangel fork, milk, a mangel cutter, and from a milk stool. According to Diagram C, Escherichia coli has been recovered from a milk sheet blotter, a beet pulp shovel, a hay truck, manger scrapings and from the rafters in a barn.

DIAGRAM A

Aerobacter oxytocum--atypical--(Migula) Bergey et al (3) (2)

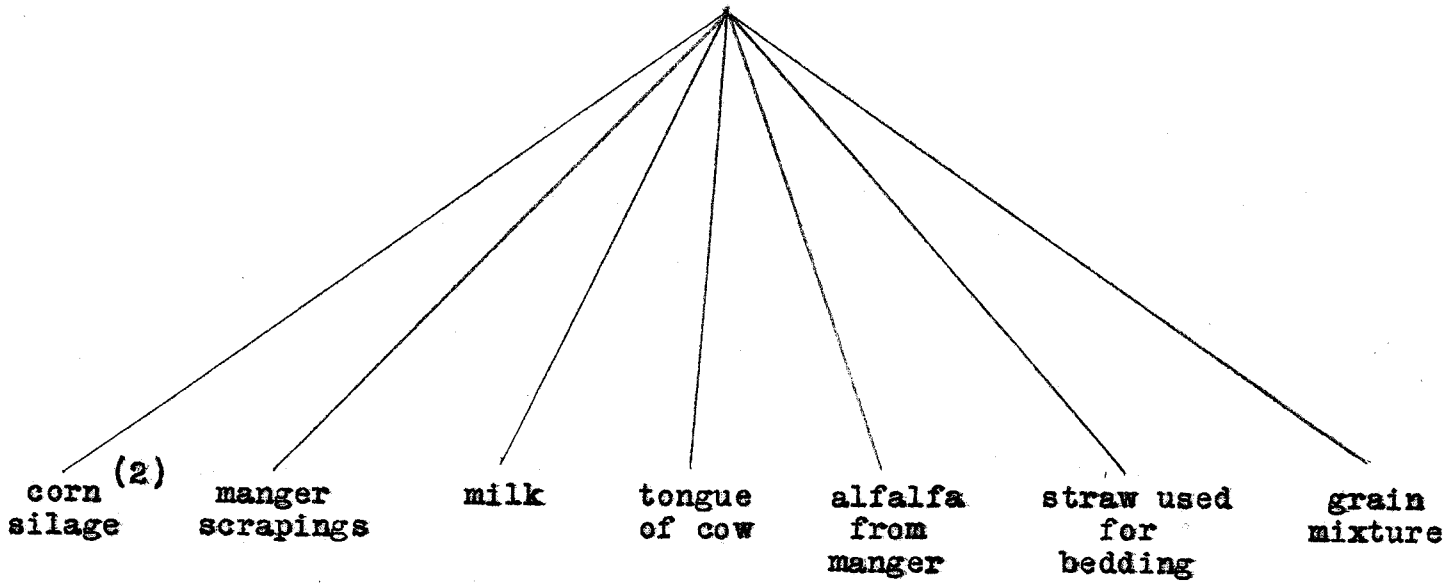


DIAGRAM B

<u>Aerobacter aerogenes</u> (Kruse) Beijerinck (3)

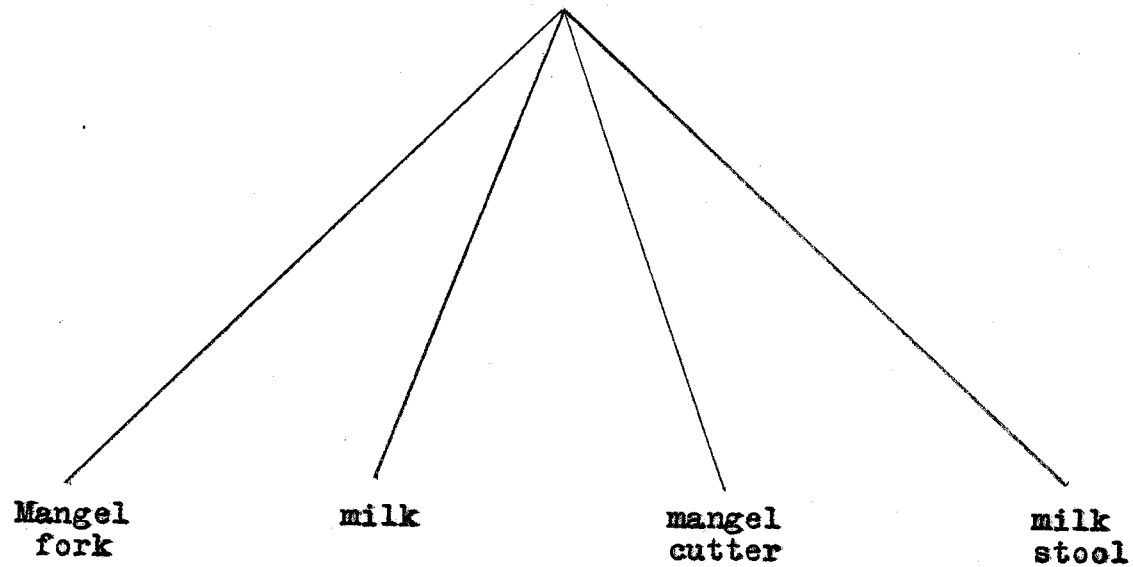
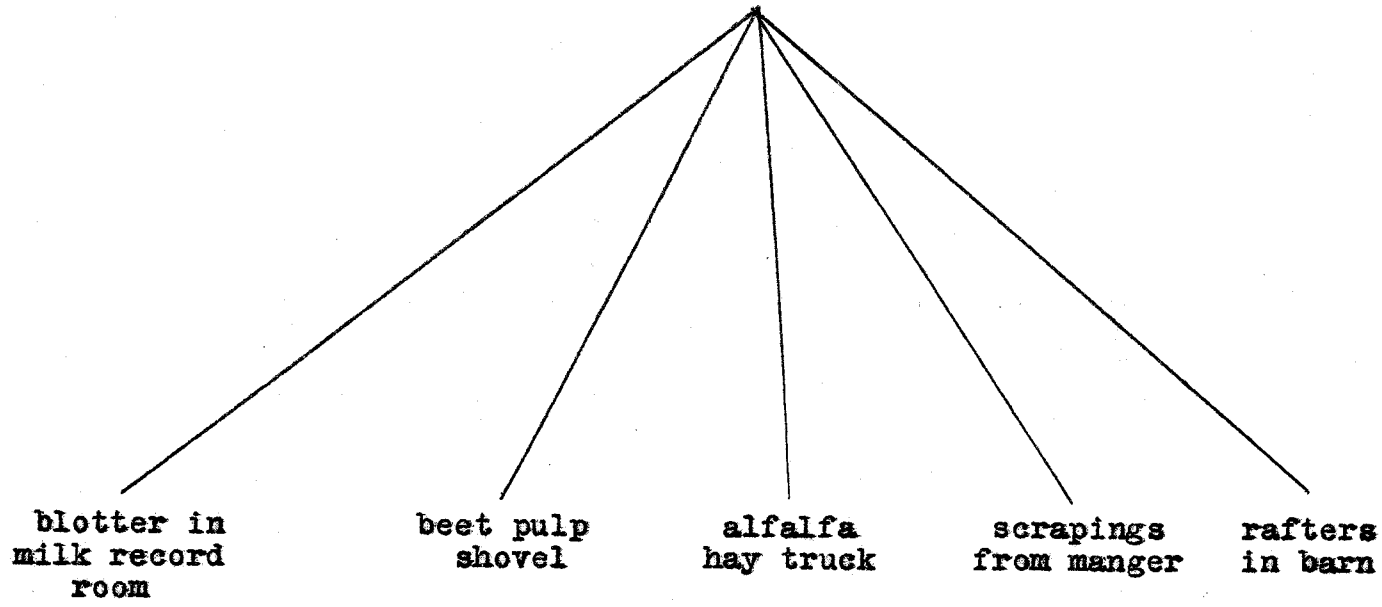


DIAGRAM C

<u>Escherichia coli</u> (Escherich) Castellani and Chalmers (3)



THE APPLICATION OF THE FINDINGS

Reviewing the work as a whole, it would seem that the findings relate themselves to the practice of milk production on the farm, to the methods in vogue in the routine inspection of dairy farms, to the management and control of milk, and to the public health aspect of milk production.

The study has shown definitely that this co-called feed flavour and stable odour in milk can be caused by specific strains of bacteria within the Escherichia-Aerobacter group, and that these strains may be obtained from numerous and varied sources. This study has demonstrated possible channels through which milk might become infected. Consequently, if the defect is to be avoided, it is necessary to employ the most approved methods and practices in the production, management and control of milk. Whilst the using of any particular feed for milk producing cows is not condemned, it will be observed that the greatest care must be exercised in management so that no bacterial contamination from the feed is possible.

This investigation has shown that the presence or absence in milk of the bacteria of the Escherichia-Aerobacter group may be taken as a measure of the conditions and the

quality of management prevailing on milk producing farms and in milk distributing depots. Possibly therefore, in the light of the results of this study, the whole question of milk control, of farm inspection and of the grading of dairy farms for milk production might to advantage be reviewed.

SUMMARY

The relation of bacteria of the Escherichia-Aerobacter group to an alleged feed flavour and stable odour in milk has been investigated.

Bacteria have been isolated from milk, feeds and farm premises.

One hundred and four organisms that were found to cause the characteristic feed flavour and stable odour in milk have been subjected to a detailed cultural study.

The respective sources of the bacteria recovered and the complete results of the detailed cultural study are summarized in Table I.

A critical analysis of the data in Table I is presented.

Based on the sum of the characteristics of the bacteria under study, one hundred and two of the one hundred

and four have been classified. Fifty-eight strains are placed within the genus Aerobacter; for all but five the species has been defined and the classified organisms include strains of Aerobacter oxytocum, Aerobacter oxytocum atypical, Aerobacter aerogenes, Aerobacter aerogenes atypical, and variants of Aerobacter cloacae, as can be seen from Table I. Forty-four of the organisms are placed in the genus Escherichia and for forty-two of the organisms the species has been defined and the classified organisms include strains of Escherichia coli, Escherichia enterica, adonite fermenting strains of Escherichia enterica, indol producing variants of Escherichia formica, Escherichia vesiculiformans, Escherichia communior, non salicin fermenting strains of Escherichia communior, Escherichia neapolitana atypical, and non salicin fermenting strains of Escherichia neapolitana--Table I.

Not only have observations been made on the intensity of the flavour and odour produced in milk by the bacteria when freshly isolated, but also on the intensity after the same organisms had been held in artificial media for some six months. Certain cultures are consistent in that they produce--to a greater or lesser degree--the characteristic feed flavour and stable odour throughout. On the whole, the bacteria classified as within the genus Aerobacter, produce a more pronounced feed flavour and stable

odour in milk than do the organisms finding themselves within the genus Escherichia.

The possible channels through which infection of the milk by the bacteria may take place are indicated pictorially.

Attention is drawn in the application of the findings to the practice of milk production, to the routine inspection of dairy farms, the management and control of milk, and to the public health aspect of milk production.

ACKNOWLEDGEMENTS

To Professor H. M. King and Professor Wilfrid Sadler for their guidance, encouragement, and advice; to the National Research Council of Canada for the funds that have made this investigation possible; and to the University of British Columbia for the laboratory facilities enjoyed, we give our most appreciative thanks.

REFERENCES

1. Sadler, Wilfrid, Irwin, M. Lenora, and Golding, N. S., 1929, December. An Alleged "Feed" Flavour in Milk Caused by Specific Bacteria. The Milk Dealer.

2. Sadler, Wilfrid, and Irwin, M. Lenora, 1930. Feed Flavour or Stable Odour in Milk Caused by an Atypical Strain of Aerobacter oxytocum. Can. Jour. of Res. 3, 200-204.

3. Bergey, D. H., 1930. Manual of Determinative Bacteriology, 3rd edition. Williams and Wilkins, Baltimore.

4. Durham, 1901. (cited by Levine, Weldin and Johnson) Jour. Inf. Diseases. 21, 1.

5. Levine, Max, Weldin, J. C., and Johnson, Beryl R., 1917. The Voges-Proskauer and Correlated Reactions of coli-like Bacteria. Jour. Inf. Diseases. 21, 1.

6. Johnson, B. R., and Levine, Max, 1917. Characteristics of coli-like microorganisms from the Soil. Jour. Bact. II, 4.

7. Jordan, E. O. 1890. Rept. Mass. State Bd. Health, Part II.

TABLE I

CULTURE NUMBER	SOURCE OF BACTERIA: FARM NUMBER	SOURCE OF BACTERIA: SPECIFIC SOURCE OF BACTERIA	V.P. M.R.	GELATINE LIQUEFACTION	MOTILITY	INDOL	DEXTROSE	LACTOSE	SUCROSE	DULCITE	SALICIN	GLYCERINE	MANNITE	SORBITE	ADONITE	XYLOSE	ARABINOSE	RHAMNOSE	LAEVULOSE	MANNOSE	GALACTOSE	MALTOSE	RAFFINOSE	DEXTRIN	INULIN	STARCH	AESCULIN	REDUCTION OF NITRATES	** LITMUS MILK 24 HOURS	CLASSIFICATION	CULTURE NUMBER
* 161	VI	MILK	POS. ?	NON-LIQ.	NON-MOTILE	FORMED.	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	NITRATES REDUCED	G	Aerobacter oxytocum	161
162	-	"	" ?	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	G	"	162
163	-	"	" ?	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	G	"	163
164	-	"	" ?	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	G	"	164
165	-	"	" ALK.	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	G	"	165
206	III	STRAW BEDDING	" ACID	"	"	NOT FORMED	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	"	AG	"	206
24	I	MANGER SCRAPINGS	" ALK.	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	Aerobacter oxytocum atypical	24
249	VII	MILK	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	G	"	249
261	-	"	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	G	"	261
265	II	TONGUE OF COW	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	265
275	-	ALFALFA FROM MANGER	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	275
276	-	STRAW BEDDING	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	276
277	-	"	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	277
282	-	GRAIN MIXTURE	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	282
283	-	"	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	283
49	I	MANGEL FORK	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	Aerobacter aerogenes	49
51		MILK	" ?	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	G	"	51
137	I	MANGEL CUTTER	" ACID	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	137
139	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	139
198	III	MILK STOOL	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	198
109	I	MIXED GRAIN IN BIN	" ALK.	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	Aerobacter aerogenes atypical	109
110	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	110
112	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	112
113	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	113
114	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	114
115	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	115
122	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	"	122
66	-	MANGEL FORK	" ?	"	"	"	AG	AG	AG	A	AG	A	AG	AG	A	AG	AG	AG	A	AG	AG	A	A	A	AG	AG	A	"	AG	Aerobacter	66
68	-	"	" ALK.	"	"	"	AG	AG	AG	A	AG	A	AG	AG	A	A	A	A	A	AG	AG	A	A	A	AG	AG	A	"	AG	"	68
127	"	MILK	" ?	"	MOTILE	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	A	A	A	AG	AG	A	A	AG	AG	"	G	"	127
213	III	MANGER SCRAPINGS	" ?	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	A	AG	AG	AG	"	AG	"	213
251	IV	BRUSH IN BARN	" ?	"	"	"	AG	AG	AG	A	AG	—	AG	—	—	A	AG	AG	AG	AG	A	AG	AG	—	AG	AG	AG	"	AG	"	251
34	I	MIXED GRAIN IN TRUCK	" ALK.	LIQ.	NON-MOTILE	FORMED	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	AG	"	AG	Aerobacter cloacae (1)	34
53	-	PIECE OF CUT MANGEL	" ?	"	"	NOT FORMED	AG	AG	AG	AG	AG	—	AG	AG	—	A	A	AG	AG	AG	AG	A	AG	—	A	AG	AG	"	AG	"	53
67	"	MIXED GRAIN IN TRUCK	" ?	"	"	FORMED	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	AG	"	AG	"	67
117	"	TONGUE OF COW	" ?	"	"	NOT FORMED	AG	AG	AG	AG	AG	A	AG	AG	—	A	A	AG	AG	AG	AG	A	AG	—	A	AG	AG	"	AG	"	117
118	"	"	" ?	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	A	A	AG	AG	AG	AG	A	AG	—	A	AG	AG	"	AG	"	118
119	"	"	" ?	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	A	A	AG	AG	AG	AG	A	AG	—	A	AG	AG	"	AG	"	119
121	"	"	" ?	"	"	"	AG	AG	AG	A	AG	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	AG	AG	"	AG	"	121
166	III	MILK	" ALK.	"	"	"	AG	AG	AG	—	AG	—	A	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	—	"	AG	"	166
167	"	"	" "	"	"	"	AG	AG	AG	—	AG	—	A	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	—	"	AG	"	167
168	"	"	" "	"	"	"	AG	AG	AG	—	AG	—	A	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	—	"	AG	"	168
169	"	"	" "	"	"	"	AG	AG	AG	—	AG	—	A	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	—	"	AG	"	169
170	"	"	" "	"	"	"	AG	AG	AG	—	AG	—	A	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	—	"	AG	"	170
188	"	FLOOR IN FEED ROOM	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	AG	"	AG	"	188
229	IV	MUD ON ROAD	" ?	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	A	AG	AG	AG	AG	AG	A	AG	—	AG	AG	AG	"	G	"	229
72	I	MANGER SCRAPINGS	" ALK.	"	MOTILE	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	—	A	AG	AG	"	AG	Aerobacter cloacae (2)	72
19	"	MANGEL FORK	" "	"	"	"	AG	AG	AG	A	AG	A	AG	AG	—	A	A	A	AG	AG	AG	AG	AG	—	A	AG	AG	"	AG	"	19
47	"	TONGUE OF COW	" "	"	"	"	AG	AG	AG	A	A	—	AG	—	—	AG	AG	AG	AG	AG	AG	AG	—	—	A	AG	—	"	AG	"	47
71	"	MANGER SCRAPINGS	" "	"	"	"	AG	AG	AG	—	AG	—	AG	—	—	AG	AG	AG	AG	AG	AG	AG	—	—	A	AG	AG	"	AG	"	71
197	III	MILK STOOL	" "	"	"	"	AG	AG	AG	—	A	—	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	—	"	AG	"	197
209a	"	MIXED GRAIN	" "	"	"	"	AG	AG	AG	—	AG	—	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	AG	AG	"	AG	"	209a
209b	"	"	" "	"	"	"	AG	AG	AG	—	AG	—	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	AG	AG	"	AG	"	209b
32	I	WATER FROM DRINKING BOWL	" "	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	AG	AG	AG	AG	AG	AG	A	AG	—	AG	AG	AG	"	AG	"	32
52	-	PIECE OF CUT MANGEL	" ?	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	A	A	AG	AG	AG	AG	A	AG	—	A	AG	AG	"	G	"	52
116	-	TONGUE OF COW	" ?	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	A	AG	AG	AG	AG	AG	A	AG	—	AG	AG	AG	"	AG	"	116
120	"	"	" ?	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	A	AG	AG	AG	AG	AG	A	AG	—	AG	AG	AG	"	AG	"	120
220	III	WALL IN BARN	" ALK.	"	"	"	AG	AG	AG	AG	AG	—	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	—	"	AG	"	220
20	I	MANGEL FORK	" ?	"	NON-MOTILE	"	A	AG	AG	A	AG	A	AG	—	—	—	A	A	AG	AG	AG	A	AG	—	A	AG	A	NOT REDUCED	AG		20
45	-	BLOTTER FOR MILK SHEET	NEG. ACID	NON-LIQ.	MOTILE	FORMED	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	AG	NITRATES REDUCED	AG	Escherichia coli	45
46	-	"	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	46
54	-	BEET PULP SHOVEL	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	—	—	AG	AG	AG	"	AG	"	54
56	-	BLOTTER FOR MILK SHEET	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	—	"	AG	"	56
58	"	HAY TRUCK	" "	"	"	"	AG	AG	A	AG	AG	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	AG	"	AG	"	58
64	"	"	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	AG	"	AG	"	64
144	-	MANGER SCRAPINGS	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	—	—	A	AG	—	"		"	144
240	IV	RAFTERS IN BARN	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	A	AG	AG	AG	AG	—	—	A	A	AG	"	AG	"	240
12	I	MILK	" "	"	NON-MOTILE	"	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	—	—	A	AG	—	"	G	Escherichia Enterica (3)	12
123	-	"	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	—	—	A	AG	—	"		"	123
263	V	"	" "	"	"	"	AG	AG	A	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	A	—	A	A	—	"	AG	Escherichia Enterica	263
40	I	BALED ALFALFA HAY	" "	"	MOTILE	"	AG	AG	A	AG	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	Escherichia formica (3)	40
43	-	BEET PULP SHOVEL	" "	"	"	"	AG	AG	A	A	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	AG	"	AG	"	43
44	-	"	" "	"	"	"	AG	AG	A	AG	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	44
57	"	BLOTTER FOR MILK SHEET	" "	"	"	"	AG	AG	A	AG	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	57
181	III	FLOOR BEHIND STALLS	" "	"	"	"	AG	AG	A	A	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	181
192	-	MANURE	" "	"	"	"	AG	AG	A	AG	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	192
194	-	"	" "	"	"	"	AG	AG	A	AG	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	194
233	IV	FLOOR BEHIND STALLS	" "	"	"	"	AG	AG	A	A	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	233
236	-	GUTTER	" "	"	"	"	AG	AG	A	A	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	236
245	-	MUD NEAR BARN	" "	"	"	"	AG	AG	A	A	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	A	—	A	A	AG	"	AG	"	245
175	III	MUD	" "	"	"	"	AG	AG	A	AG	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	AG	"	AG	"	175
267	II	STANCHIONS	" "	"	"	"	AG	AG	A	A	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	A	—	"	AG	"	267
269	-	MANURE	" "	"	"	"	AG	AG	A	A	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	A	—	"	AG	"	269
61	I	FLOOR WHERE GRAIN IS MIXED	" "	"	NON-MOTILE	"	AG	AG	A	AG	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	Escherichia vesiculi-formans	61
63	-	"	" "	"	"	"	AG	AG	A	AG	A	A	AG	AG	—	A	A	A	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	63
4	-	MILK	" "	"	MOTILE	"	AG	AG	AG	AG	AG	—	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	—	AG	AG	—	"	AG	Escherichia communior	4
16	-	"	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	—	—	AG	AG	A	"	G	"	16
17	-	"	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	—	—	AG	AG	AG	"	G	"	17
85	-	"	" "	"	"	"	AG	AG	AG	AG	AG	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	AG	"	G	"	85
145	-	"	" "	"	"	"	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	—	—	—	AG	A	"	G	"	145
130	-	MUD IN PADDOCK	" "	"	"	"	AG	AG	AG	A	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	Escherichia communior (3)	130
146	-	MILK	" "	"	"	"	AG	AG	AG	AG	A	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	AG	"		"	146
219	III	DITCH ON ROAD	" "	"	"	"	AG	AG	AG	AG	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	219
50	I	GUTTER	" "	"	NON-MOTILE	"	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	"	AG	Escherichia neapolitana atypical	50
21	-	MANGEL CUTTER	" "	"	"	NOT FORMED	AG	AG	AG	A	AG	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	AG	"	AG	"	21
22	-	"	" "	"	"	"	AG	AG	AG	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	A	AG	AG	AG	"	AG	"	22
28	"	WET BEET PULP	" "	"	"	FORMED	AG	AG	AG	AG	A	—	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	—	"	AG	Escherichia neapolitana (3)	28
41	"	TONGUE OF COW	" "	"	"	"	AG	AG	AG	AG	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	AG	"	AG	"	41
39	"	BALED ALFALFA HAY	" "	"	"	"	AG	AG	AG	AG	—	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	A	A	—	"	AG	"	39
99	-	MILK	" "	"	"	"	AG	AG	AG	AG	—	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	—	"		"	99
222	IV	"	" "	"	"	"	AG	AG	AG	AG	A	A	AG	AG	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	—	"	G	"	222
13	I	"	" "	"	MOTILE	NOT FORMED	AG	AG	AG	—	—	—	AG	—	—	A	A	A	A	A	A	A	A	—	AG	AG	—	"	G	Escherichia	13
255	IV	WOODEN STANCHIONS	" "	"	NON-MOTILE	"	AG	AG	AG	—	A	—	AG	—	—	A	AG	AG	AG	AG	AG	AG	AG	—	AG	AG	—	"	AG	"	255
212	III	MANGER SCRAPINGS	" "	"	"	"	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	—	A	A	A	NOT REDUCED	AG		212

FARM NUMBER
I THE UNIVERSITY FARM POINT GREY B.C.
II A FARM ON VANCOUVER ISLAND "
III " " " LULU ISLAND "
IV " " " NICOMEN ISLAND "
V " " AT MISSION "
VI " " " HARRISON MILLS "
VII " " " LADNER "

*ALL CULTURES GRAM NEGATIVE RODS.
AG ACID AND GAS — MEDIA SPLIT IN LAYERS, AND BLOWN UP TUBE BY GAS.
AG ACID AND GAS — GAS PRODUCTION PROFUSE.
ag ACID AND GAS.
A ACID NO GAS.
— NO ACID AND NO GAS.

(1) CLASSIFIED AS NON-MOTILE VARIANTS OF AEROBACTER CLOACAE.
(2) " " " VARIANTS OF AEROBACTER CLOACAE.
(3) WITH QUALIFICATIONS: SEE TEXT.

** A DISCUSSION ON THE PRODUCTION BY THE BACTERIA OF THE ALLEGED FEED FLAVOR AND STABLE ODOUR IN MILK IS TO BE FOUND IN THE TEXT PAGES:

Employing Simmon's Citrate Agar all organisms within the genus Aerobacter brought about a blue color throughout the medium. Three strains, cultures 50, 21, and 22 within the genus Escherichia also brought about a blue color throughout the medium.