THE SANITARY SIGNIFICANCE OF PECTIN-FERMENTING, LACTOSE-FERMENTING, GRAM-NEGATIVE, NON-SPORE-FORMING BACTERIA IN WATER

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Although numerous workers have shown that gram-negative, non-spore-forming, lactose-fermenting, pectin-fermenting bacteria are widely distributed in nature, their sanitary significance remains uncertain. Bergey’s Manual of Determinative Bacteriology (1939) describes the tribe Erwineae as plant parasites causing blights and soft rots, fermenting glucose and lactose with the formation of acid, or acid and a small amount of visible gas, and usually attacking pectin. Since these organisms resemble closely the coli-aerogenes group, those which produce visible gas might be expected to pass through the completed test in the bacteriological examination of water, and give false tests. Whether this group of plant pathogens can be differentiated from coli-aerogenes organisms of intestinal origin by their ability to ferment pectin cannot be stated until the occurrence of pectin-fermenting coli-aerogenes organisms in the intestinal tract of man and animals has been investigated. This study has been undertaken in order to determine: (1) a suitable medium for the testing of the ability of organisms to ferment pectin; (2) the incidence of pectin-fermenting, lactose-fermenting, gram-negative, non-spore-forming bacteria in fecal material of man and animals; (3) the incidence of the above organisms in water; and (4) the cultural, morphological and biochemical characteristics of pectin-fermenting bacteria from the feces of man and animals, and from water.

Kruse (1910) referred to many investigators who had designated such common organisms as Bacillus subtilis and Escherichia coli as pectin-fermenting bacteria. Coles (1928) found that citrus pectin (heat sterilized) was fermented by seven out of thirteen strains of Bacterium oxytocum, four out of nine strains of Bacterium aerogenes and two strains of Bacillus aceto-ethylicum. He concluded that only organisms which commonly occur in the soil are capable of attacking pectin with the production of acid and gas. He also stated that none of the intestinal forms, classified in the sub-genus Escherichia of the genus Bacterium, produced acid or gas from pectin; however, he did find that some of the organisms belonging to the sub-genus Aerobacter were able to ferment pectin with the production of acid and gas. Burkey (1928) carried out an extensive study of pectin-fermenting organisms which had been isolated from soil, creek water, decayed potatoes, parsnips, cornstalks, hay infusions and sewage. He found that all of the gram-negative, lactose-fermenting organisms which possessed the ability to ferment citrus pectin (heat sterilized) produced both acid and gas from it. All of these organisms had characteristics similar to those of coliform organisms, including the ability to form typical colonies on Endo and eosin-methylene-blue agars.

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He classified all of them in the genus *Aerobacter* but drew no conclusions as to their sanitary significance.

Werch, Day, Jung, and Ivy (1941), in a study of the role of the intestinal bacteria in the decomposition of pectin, used a broth containing a one-per-cent solution of pure citrus pectin which had been sterilized by filtration. Although their work dealing with the isolation of the responsible bacteria, with the determination of the exogenous or endogenous source of the pectinase and with the products of decomposition was not completed, they concluded that pectin is decomposed by bacteria present in the feces.

The ability of the various species of *Erwinia* to ferment pectin is too well known to require reviewing.

**EXPERIMENTAL**

1. Development of suitable media for testing the ability of organisms to ferment pectin

Chemically pure apple pectin obtained from the General Foods Sales Company, New York, was used in this investigation. This pectin was shown to be free from reducing substances other than pectin by two methods. In the first method a 70 per cent ethyl alcohol extract was tested with Benedict's solution; in the second method the pectin was precipitated from a 2 per cent solution and the filtrate was dealed with hydrogen sulfide and tested with Benedict's solution. Controls containing small amounts of arabinose and galactose gave positive qualitative Benedict tests by both methods.

Since no definite information could be found concerning the effect on pectin of sterilization by heat, the effect of autoclaving a one per cent solution was investigated. At pH 5.0, pH 6.0 and pH 6.5, the pectin was hydrolized to reducing substances. At pH 7.0 only traces of reducing substances were produced, but at pH 7.2 and pH 7.5 large amounts of reducing substances were again produced.

Since from these experiments it was concluded that it would be unsafe to test the ability of organisms to ferment pectin using a pectin-containing medium which had been sterilized by heat, the sterilization of the pectin was carried out in the following manner. Five grams of pectin were added, under aseptic conditions, to a sterile one-liter Erlenmeyer flask. To this were added 20 ml. of 75 per cent ethyl alcohol, under aseptic conditions, and the flask was rotated rapidly to mix the contents. Since a high concentration of alcohol was undesirable in the final medium it was necessary to allow the alcohol to evaporate from the sterilized pectin before proceeding to the next step. It was found that, if the flask was allowed to remain at room temperature for 5–8 days, the alcohol would evaporate from the flask and leave the pectin with just a thin coating of alcohol. This coating of the grains of pectin with alcohol proved to be desirable for, when 500 ml. of sterile distilled water were added to the flask, the pectin dispersed into the water at once, giving a one-per-cent solution which was clear and free of clumps. This one-per-cent solution could be added to any basal medium aseptically and used for the determination of the ability of organisms to ferment pectin. In this investigation a basal medium containing 4 grams of ammonium
chloride and 4 grams of dibasic potassium phosphate per liter of distilled water, and one containing 6 grams of Bacto beef extract and 10 grams of peptone per liter of distilled water, were used. It was necessary to prepare these basal media in the above double strengths and to so adjust the pH of each that, after the addition of an equal quantity of the alcohol-sterilized pectin solution, the desired concentration of the ingredients would be obtained and the final pH of each medium would be 7.1. A combination indicator containing brom-cresol-purple and cresol red was used. Two milliliter amounts of the basal medium were placed in Durham fermentation tubes and sterilized, after which 2 ml. amounts of sterilized pectin solution were added aseptically. After the addition of the pectin, the tubes of media were placed in the incubator at 37° C. for 48 hours and then at room temperature for 4 days, in order to detect contamination.

Since, throughout these studies, neither acid nor gas production was ever observed in the extract pectin broth, except for acid production in check Erwinia cultures, the results with this medium will be omitted from the tabulations. As acid was frequently produced in duplicate inoculations in the synthetic medium, it would appear that fermentation was probably masked in the extract broth.

2. The incidence of pectin-fermenting, lactose-fermenting, gram-negative, non-spore-forming bacteria in human and animal feces

Eosin methylene-blue agar streak plates were made from feces samples from 56 humans, 7 cows, 3 mules and 7 dogs and from the caecal contents of 6 fowls. After a 48 hr. incubation period well isolated colonies were picked from the plates into duplicate tubes of both extract pectin broth and synthetic pectin medium. Two or more colonies of each type present were picked from each plate. Transfers were made from all colony types which, if they had appeared on a plate from water, would have been considered to be typical or atypical colonies of coliform organisms. All cultures so isolated will be referred to as strains, even though many of the cultures were apparently duplicates. Controls consisted of uninoculated tubes and of duplicate tubes inoculated with a known pectin-fermenting Erwinia and with a known non-pectin-fermenting fecal strain of Escherichia coli. The number of samples studied, their source, the number yielding pectin-fermenting, lactose-fermenting, gram-negative, non-spore-forming bacteria, the number of strains tested for ability to ferment pectin, and the number which fermented synthetic pectin medium with the production of acid are included in table 1.

None of 507 strains of organisms which were isolated from 56 human stools fermented pectin. Gram-negative, lactose-fermenting, non-spore-forming organisms with the ability to form acid in the synthetic pectin medium were isolated from the feces of one of seven cows, one of three mules, and from four of seven dogs. None were isolated from the samples from three horses and six fowls. In all, of 477 strains which were isolated from animal feces, only 48 produced acid in the synthetic pectin medium. None produced gas. Thirty-eight of the 48 strains were from dogs. It is possibly significant that the dogs were laboratory animals which had been fed only on prepared chow. The majority of the 48 strains of pectin fermenting organisms were from colonies which
resembled those of *Aerobacter*; none were from colonies which resembled those of typical *Escherichia*. However, not all of the organisms which produced aerobacter-like colonies were able to ferment pectin.

All of the three strains which had been isolated from cow feces proved to be methyl-red positive. Two gave the “Imvic” reactions + + + + and appeared to be *Escherichia* intermediates. The other, when first isolated, produced gas and acid from lactose. However, when its ability to ferment lactose was tested for the second time, using carefully sterilized lactose broth, it failed to produce gas. This may have been due either to the loss of the ability to produce gas, or to the lactose broth used in the first test having been injured by the process of sterilization. This strain gave the “Imvic” reactions − + − −, and appeared to be an *Escherichia* organism. Four strains which had been isolated from mule feces proved to be methyl-red negative and Voges-Proskauer positive. The “Imvic” reactions − − + + were obtained with three of the strains, and + + + +

### Table 1

The incidence of gram-negative, pectin-fermenting, lactose-fermenting, non-spore-forming bacteria in human and animal feces

<table>
<thead>
<tr>
<th>Source of Samples</th>
<th>No. of Samples Examined</th>
<th>No. of Samples Yielding Pectin Fermenters</th>
<th>No. of Strains Picked from E.M.B. Plates and Tested for Ability to Ferment Pectin</th>
<th>No. of Strains Which Fermented Pectin (Synthetic Medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans</td>
<td>56</td>
<td>0</td>
<td>507</td>
<td>0</td>
</tr>
<tr>
<td>Cows</td>
<td>7</td>
<td>1</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>Horses</td>
<td>3</td>
<td>0</td>
<td>68</td>
<td>0</td>
</tr>
<tr>
<td>Mules</td>
<td>3</td>
<td>1</td>
<td>44</td>
<td>7</td>
</tr>
<tr>
<td>Dogs</td>
<td>7</td>
<td>4</td>
<td>155</td>
<td>38</td>
</tr>
<tr>
<td>Fowls</td>
<td>6</td>
<td>0</td>
<td>118</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>82</td>
<td>6</td>
<td>984</td>
<td>48</td>
</tr>
</tbody>
</table>

with one. All of these appear to be *Aerobacter* strains. The characteristics of three strains which, on preliminary examination, appeared to be identical with some of the strains which were studied, were not investigated in detail. All of the 38 strains which were isolated from dog feces gave the “Imvic” reactions − − + +, and appeared to be typical strains of *Aerobacter aerogenes*. All of the strains which were motile showed a peritrichous arrangement of flagella.

The abilities of the 48 pectin-fermenting strains to ferment lactose, sucrose, glucose, maltose, alpha-methyl-glucoside, cellobiose, levulose, mannose, galactose, raffinose, rhamnose, trehalose, melezitose, salicin, amygdalin, aesculin, xylose, arabinose, glycerol, mannitol, dulcitol, sorbitol, adonitol, inositol, erythritol, glycogen, dextrin, inulin and soluble starch were determined. Their fermentation characteristics proved to be of little value in their identification.\(^1\)

\(^1\) For results of fermentation tests see The Sanitary Significance of Pectin-fermenting, Lactose-fermenting, Gram-negative, Non-spore-forming Organisms.—D. B. McFadden, Thesis, University of Kentucky.
3. The incidence of pectin-fermenting, lactose-fermenting, gram-negative, non-spore-forming bacteria in water

For the attempted isolation of pectin-fermenting coliform organisms from water, samples from a number of different sources were collected according to the Standard Methods of Water Analysis, 1936, and taken to the laboratory where they were examined as quickly as possible. As this study of pectin-fermenting coliform organisms in water was made with the view not only to determine the incidence of these organisms in water, but also to determine if colonies of these organisms would appear on eosin methylene-blue plates following the usual procedure of enriching water samples in lactose broth, the following method for the isolation of these organisms was selected.

Duplicate tubes containing 10 ml. amounts of Standard Methods double-strength lactose broth were inoculated with 10 ml. portions of the water sample, and duplicate tubes of single-strength Standard Methods lactose broth were inoculated with 1.0 ml. and 0.1 ml. portions of the sample. The contents of the tubes which showed production at the end of 48 hours were streaked on Standard Methods eosin methylene-blue agar and, after incubation for 48 hours, well isolated colonies were picked from the plates to nutrient agar slants. At least two colonies of each type present on the plates were picked. Each culture thus obtained was considered to be a strain. Since the incidence of pectin-fermenting organisms in water was not known, enrichment with pectin broth was also used as an aid in the isolation of these organisms from the first samples which were examined. Ten milliliters of the sample were placed in duplicate tubes of double-strength extract pectin broth and double-strength synthetic pectin medium. The concentration of pectin in these media was one per cent and each tube contained 10 ml. of the medium. One milliliter and one tenth milliliter portions of the sample were placed in duplicate tubes of single-strength extract pectin broth and synthetic pectin medium. The contents of tubes which showed the production of acid, or acid and gas after 7 days incubation were streaked out on eosin methylene-blue agar plates, and well isolated colonies were picked from these plates after 48 hours' incubation. All of the strains which were isolated were tested for their ability to ferment pectin and lactose.

Eleven samples of water were taken from ponds, wells, a cistern, a lily pool, a creek and a boiler compound, and examined for the presence of pectin-fermenting, lactose-fermenting, gram-negative, non-spore-forming bacteria. The results are presented in table 2.

From table 2 it can be seen that all of the samples except three yielded gram-negative, lactose-fermenting, pectin-fermenting, non-spore-forming bacteria. Of 259 strains which were isolated from the eosin methylene-blue plates, 71 produced acid from pectin and acid and gas from lactose. Thirty-five of these strains were isolated following pectin enrichment and 36 following lactose enrichment. All of the 71 strains would have been isolated by the Standard Methods of Water Analysis procedure and would have been considered to belong to the coli-aerogenes group.
Detailed characteristics of 51 of the 71 gram-negative, lactose-fermenting, pectin-fermenting, non-spore-forming organisms which were isolated from water have been studied. Nineteen, which were obviously duplicates of some of those that have been studied, were given only preliminary study. The 51 strains fall into eleven groups according to their "Imvic" reactions. In the first group are four strains, with the reactions + + − −, which appear to be typical strains of Escherichia coli. The second group consists of six strains, with the reactions − + − −, which appear to be indole-negative Escherichia. The next three groups appear to be Escherichia intermediates. In the third group are ten strains with the reactions + + + −, in the fourth four strains with the reactions − + + + and in the fifth two with the reactions + + + +. Of thirteen strains in the sixth group, with the reactions − − + +, eleven have the characteristics of Aerobacter aerogenes and two the characteristics of Aerobacter cloacae. In group seven are six strains, with the reactions + − + +, which might be considered to be indole-producing strains of Aerobacter. The one strain in group eight gives the reactions − − + −. Although the fermentation characteristics of the strains in the remaining groups are typical of the coli-aerogenes group, the "Imvic" reactions make their relationships uncertain. In group nine are three strains with the reactions − − − +, in group ten one strain with the reactions + − + + and in group eleven one strain with the reactions − − − −.  

\*\*\*For results of fermentation tests see The Sanitary Significance of Pectin-fermenting Lactose-fermenting, Gram-negative, Non-spore-forming Organisms.—D. B. McFadden, Thesis, University of Kentucky.\*\*\*
DISCUSSION

Burkey (1928) and Coles (1928) reported that some strains of coliform organisms were able to produce both acid and gas from a synthetic pectin medium. On the other hand none of the strains which we have encountered produce gas from synthetic pectin medium. Since we have shown that heat sterilization produces reducing substances from pectin and since both of these investigators employed heat-sterilized media, it is possible that the gas was produced from some product which had resulted from hydrolysis of the pectin.

Extract pectin broth appears to be unsatisfactory for the detection of fermentation by coliform organisms. Apparently fermentation is masked by the production of alkali in the medium. When the pH values of extract pectin broth cultures were determined electrometrically, they were found to have increased, whereas the pH values of the synthetic pectin medium cultures had dropped to as low as pH 4.82 after seven days incubation. The strain of Erwinia which was used as a control produced acid in both media.

The strains of pectin-fermenting coliform organisms which we have isolated from animal feces and from water appear to be somewhat more varied than those which were obtained from water by Burkey. He classified all of his strains in the genus Aerobacter. Of the strains which we have isolated from animal feces, those from the cow were methyl-red-positive intermediates while those from mules and dogs were typical strains of Aerobacter aerogenes. The strains which we have obtained from water include a number which appear to be intermediates, three strains of typical Escherichia coli and many strains of Aerobacter.

We have classified all of our strains of pectin-fermenting coliform organisms in the genera Escherichia and Aerobacter although it is possible that some of the strains may belong in the genera Erwinia and Serratia. According to Bergey's Manual of Determinative Bacteriology (1939) it would appear that organisms in the genus Erwinia differ from those in the tribe Escherichae by usually fermenting pectin and by being pathogenic for plants. The results of our study of pectin-fermenting organisms seem to point out that the ability to ferment pectin is possessed by a great many members of the coliform group and that, therefore, this characteristic is not of much value in identifying members of the genus Erwinia. No complete record of the characteristics of the members of the genus Erwinia is available. Without knowledge of the reactions of these organisms on the media which are commonly used for the study of organisms in the Escherichae group it does not appear to be possible to determine if any of the coliform organisms which are obtained from water are Erwinia.

Pederson and Breed (1928) in a study of the fermentation of glucose by organisms of the genus Serratia, came to the conclusion that non-pigmented strains of Serratia might be properly classed in the genus Aerobacter. Accordingly, some of the pectin-fermenting strains from water, which rapidly liquefy gelatin and utilize uric acid, may be non-pigmented strains of Serratia.

At present, it is not possible to state what is the sanitary significance of pectin-fermenting, lactose-fermenting, gram-negative, non-spore-forming organisms in
likely, therefore, that many of the pectin-fermenting organisms which we have isolated from water, using Standard Methods procedure, ferment pectin, whereas none from human feces and only a few from animal feces ferment pectin. It appears likely, therefore, that many of the pectin-fermenting organisms which we have isolated from water were not of fecal origin. According to the present isolated from human feces presence of the following organisms which we have studied. Pectin-fermenting ability has been tested in extract broth and synthetic bases to which alcohol-sterilized pectin had been added aseptically. Pectin-fermenting strains have been detected by the production of acid in a synthetic medium. No visible fermentation occurred in the extract broth medium.

Of 507 strains of coliform organisms which were isolated on eosin methylene-blue agar from the feces of 56 persons, 68 strains which were isolated from the feces of three horses and 118 strains which were isolated from the feces of 6 fowls, none fermented pectin. Of 92 strains which were isolated from the feces of seven cows, three, all of which were isolated from the same cow, fermented pectin. Of 44 strains which were isolated from the feces of 3 mules, 7, all of which were isolated from the same mule, fermented pectin. Of 115 strains which were isolated from the feces of 7 dogs, 38, which were isolated from 4 of the 7 dogs, fermented pectin.

In order to determine the incidence of pectin-fermenting coliform organisms in water, pectin fermentation tests have been made on strains which were obtained from colonies on eosin methylene-blue agar plates that had been made following the enrichment of water samples in Standard Methods lactose broth and in pectin enrichment broth. Of 259 strains which have been isolated, 71 have been found to ferment pectin. Of these, 35 were from plates which had been streaked from pectin enrichment media and 36 from plates which had been streaked from Standard Methods lactose broth.

Four of the pectin-fermenting strains, all of them isolated from water, possessed typical Escherichia coli characteristics, 28 strains, from mule and dog feces and from water, possessed typical Aerobacter aerogenes characteristics and 32 strains, from cow feces and from water, possessed characteristics of intermediate coliform organisms.

Due to lack of information concerning the reactions of members of the genus Erwinia and of non-pigmented strains of Serratia on the media which are used for studying coliform organisms, it has been impossible to determine if any of the pectin-fermenting organisms which have been isolated belong to either of these genera.

It has been concluded that since relatively more pectin-fermenting coliform organisms are found in water than in the feces of animals, some of them, at least, are probably not of fecal origin.

SUMMARY

The incidence of pectin-fermenting, lactose-fermenting, gram-negative, non-spore-forming bacteria in the feces of man and of animals and in water has been studied. Pectin-fermenting ability has been tested in extract broth and synthetic bases to which alcohol-sterilized pectin had been added aseptically. Pectin-fermenting strains have been detected by the production of acid in a synthetic medium. No visible fermentation occurred in the extract broth medium.

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