# STUDIES ON A BACTERIAL DISEASE OF MELONS

by

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# INTRODUCTION

In the spring of 1948 a disease mainly affecting cantaloupes, but also damaging crops of Honey Dew melons, occurred in southern Arizona. Early in the season the disease on cantaloupes threatened to assume nearly epiphytotic proportions in the Salt River Valley. It was also reported on a few cantaloupe vines in an isolated planting in Pima county. This disease is thought to be caused by a seed-borne bacterium, possibly Erwinia aroideae. Secondary factors contributing to the seriousness of the disease are believed to be damp, cool weather at the beginning of the season, the abundant presence of insect vectors, followed by exceptionally dry weather later on that both hastened water loss from the infected foliage and that was also not entirely unfavorable to the development of the bacterium already within tissues of the plants.

Previously only two diseases of bacterial origin have been verified for the cantaloupe. These are caused by Erwinia tracheiphila and Erwinia aroideae in which is included Bacillus melonis (1). This thesis describes work done in the attempt to classify the last-named parasite.

## LITERATURE

The first work of any extent on Erwinia aroideae was done by C.O. Townsend (13), and published in 1904. In his paper he mentioned the work of Halstede in 1893 who presented a brief description of a soft rot of the calla corm. Townsend's paper described the cause and general appearance of the disease; the effect of the organism on calla, and the morphological and physiological characters of the organism. He also compared the calla-rot organism with similar organisms, discussed the origin and spread of the disease, and suggested controls. He proposed that the calla-rot organism should be named Bacillus aroideae.

In 1910 N.J. Giddings (6) wrote a detailed paper on "A Bacterial Soft Rot of Muskmelon Caused by Bacillus melonis n. sp." His paper included the pathogenesis and morphological, cultural, physical, and biochemical characters of the bacillus, and suggested control measures.

Giddings' bacterium appears to be similar to that of Townsend on the calla corm. Erwin F. Smith (11) in his book "Bacterial Diseases of Plants," stated that he believed Bacillus aroideae and Bacillus melonis are identical. However, the various workers on these two bacteria do not seem to agree on their identity, as will be indicated further in

this discussion.

S.A. Wingard (16) in 1924 reported Bacillus aroideae, Townsend, to be the cause of a bacterial soft rot of tomato causing severe losses in Virginia.

A.B. Massey (8) in 1924 did further work with Wingard's (16) organism that caused a tomato soft rot. Massey compared Bacillus aroideae with Bacillus carotovorus Jones. He used both pathogenecity and laboratory cultures to distinguish between the two organisms.

Philip Brierley (2), 1928, published a paper dealing with the pathogenecity of four species of bacteria on potato tubers. Bacillus aroideae was one of the three bacteria that were used in comparison with B. mesentericus as a measure of its pathogenecity.

C. Elliot (4), 1930, listed Erwinia aroideae and Erwinia melonis separately. A morphological and cultural description of the bacteria as well as the symptoms of an infected plant and control measures, together with a list of hosts for each of the bacteria, were included.

Roger Patrick and C.H. Werkman (9), 1932-33, published on "Bacteria Fermenting Xylan." They showed that Bacillus melonis fermented xylan. However, the description of their bacterium isolated from a watermelon indicates the possibility of its being a separate strain of B. melonis.

In a bulletin concerning market diseases of cantaloupes and similar melons, James S. Wiant (15), 1937, reported a

bacterial soft rot present on the melons in one of the southeastern states and the disease as abundant on Chilean Honey Dew melons. It was believed that this soft rot was possibly due to B. aroideae or B. melonis.

R.P. Elrod (5), 1941, in his paper dealing with the soft-rot group and some of their biochemical and serological actions, considered that E. aroideae is synonymous with E. carotovora, whereas W.J. Dowson (3), whose work was mentioned in Elrod's paper, considered them as distinct species.

E.L. Waldee (14), 1944-45, placed both B. aroideae and B. melonis in the genus Pectobacterium, on the basis of their pectolytic activity. Also from results in cultural tests, he concluded that aroideae and melonis are valid species.

In the sixth edition, 1948, of Bergey's Manual of Determinative Bacteriology (1), E. aroideae and E. carotovora are listed as separate species; however, Elrod's (5) reference to their identity is quoted. E. aroideae and E. melonis are given as synonyms, and E. melonis as described by C.H. Werkman (9) is listed separately.

## SYMPTOMS

Early in the season the young cantaloupe fields, as a whole, showed numerous dark areas caused by the infection, browning and death of the leaves. These areas in the field varied in size from a single plant or small group of plants scattered irregularly to partial and occasionally to entire rows. Later in the season, however, the fields appeared green and the infected areas became inconspicuous from a distance. Upon close inspection, many of the plants still showed symptoms of infection consisting of the brown, dried remains of the leaves first attacked lying in the center of the hill, and spots on the older and new leaves of the outward developing runners. Late in the season, about July 15, the cantaloupe fields that had not yet been disked, showed predominantly dead, dried foliage and consequently sunburned fruits as compared with the greener foliage of more healthy fields. (Fig. 1).

The earliest symptom observed in the field was a small, slightly water-soaked spot either on or close to the leaf margin (Fig. 2a). In one field these spots were very water-soaked, and there was a dark ooze upon the spot. In general the spots were olive-lake (10) in color. The cotyledons often were browned and shriveled and hung down

beside the main stem. In greenhouse tests with artificial inoculations, the first symptoms were round to irregular spots on the cotyledons, buffy citrine (10) in color. Later similar spots appeared on the true leaves. The infected areas on the cotyledons rapidly increased in size, involving the whole tip and finally the entire cotyledon which then shriveled, dried and hung down beside the stem. On the true leaves, both in the greenhouse and in the field, the infected area increased in size usually in a wedge-shaped pattern with the point of the wedge towards the center of the leaf (Fig. 3c). Eventually the greater part of or the entire leaf became infected. These affected areas on the blade became dry and brittle, often jagged and torn by the action of the wind (Fig. 4d).

The leaf stem or petiole frequently was water-soaked and had depressed areas. Later the petiole became dried, shrunken and twisted (Fig. 4e). The leaves killed by the infection remained attached to the vine by this tough dried petiole. Petiolar infection seemed either to follow or be coincident with infection of the leaf. The tendrils were frequently attacked, turning brown and dying (Fig. 2b).

The buds and flowers were also infected and blighted, usually irregularly along the full length of a runner. The infected flowers turned brown and eventually dropped off.

Numerous blighted young fruits that apparently resulted from previous floral infection appeared in the fields

(Figs. 2c, 4c, 5d, 6). These fruits were discolored, first to yellow and later to a light brown. Some young fruits showed a sunken and shriveled area on the surface. The largest shriveled fruits were approximately six inches in length. The seeds from the infected fruits were normal in appearance, but were shown in culture to carry the bacterium upon their surface (Fig. 7).

Figure 1. Blighted cantaloupe field in Salt River Valley


Figure 2. Natural infection of (a) leaves, early stage, (b) tendrils, (c) young fruits


Figure 3. Upper leaf: (a) leaf-miner injury, no infection. Middle leaf: (b) leaf-miner injury plus a natural infection. Lower leaf: (c) wedge shape of an advancing natural infection.


Figure 4. Natural infection of (d) leaves; advanced stage showing drying and tearing of tissue. (e) Dried, shrunken, twisted petiole resulting from infection. (f) Naturally blighted young fruit.


Figure 5. Natural infection of (d) fruit on plant; (e) leaf-miner injury

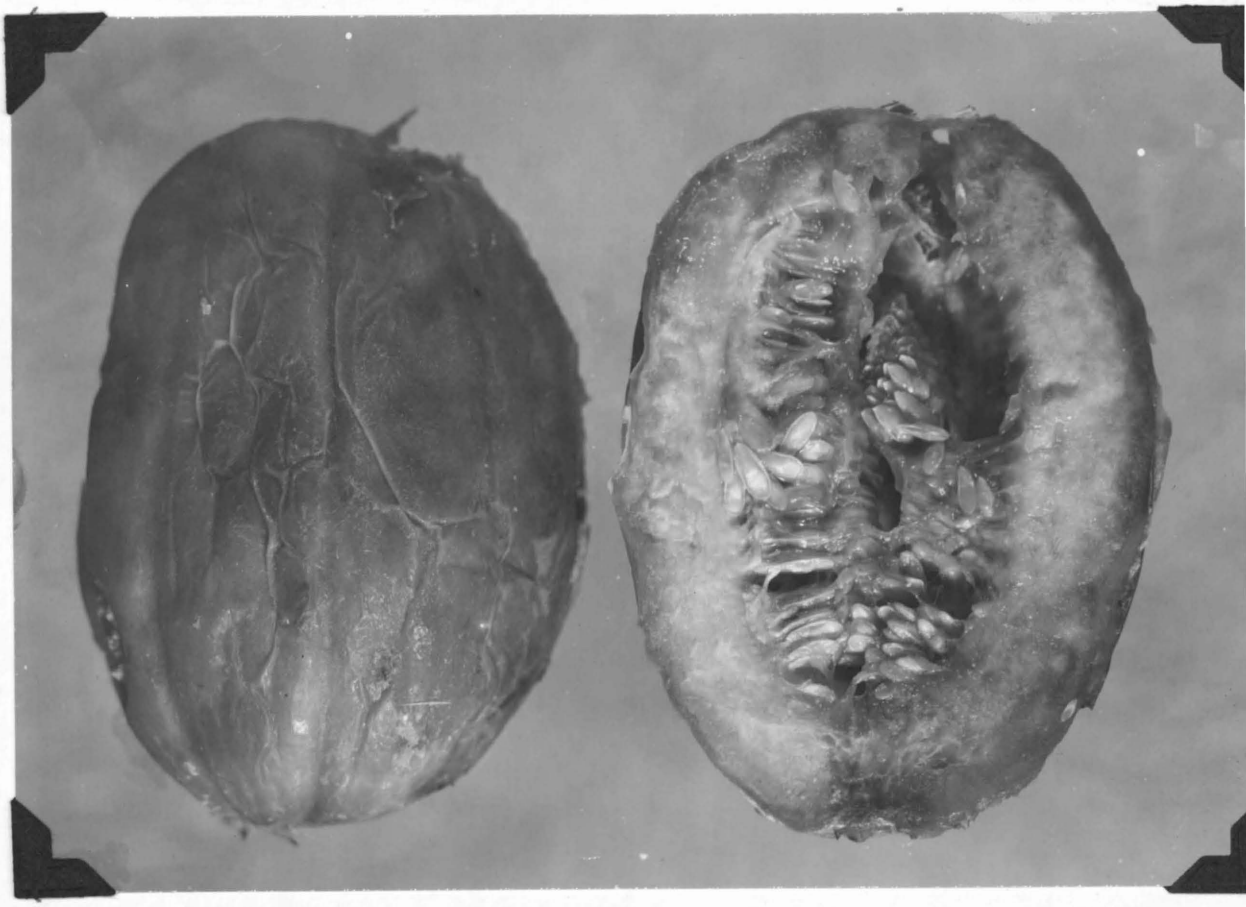

Figure 6. Naturally infected young cantaloupe fruit from Salt River Valley

## LIST OF CULTURES

1984: *Bacterium carotovorum*. Calla-NCTC. Recd. 1949

5973: *Bacterium carotovorum*, var. *melonis*. ATCC #920: NCTC. Recd. 1949

T-Z : Isolate from cantaloupe leaf: Salt River Valley: 1948

CuY : Isolate from cantaloupe leaf: Salt River Valley: 1948

C : Isolate from cantaloupe seedling: Salt River Valley: 1949

Y : Isolate from cantaloupe leaf: Yuma: 1949.

## ISOLATIONS

During the summer of 1948 and spring of 1949, attempts were made to isolate the organism causing the disease described on cantaloupe plants. Some of the material was collected on field trips to the Salt River Valley, but most of it was sent in by County Agents or brought in by members of this department.

The usual methods of cutting out a bit of the diseased tissue, surface-sterilizing it in $HgCl_2$, 1:1000, rinsing with sterile distilled water, and placing it on a poured agar plate were followed. The plant parts cultured

included leaves, petioles, young and mature fruits, and seeds. All of these yielded at least one bacterium, and often a mixture of bacteria. The bacterial growths were transferred to agar slants and then dilution plates were poured. A representative of each type of bacterial colony was transferred to an agar slant for further work.

This resulted in a large number of cultures, several of which were probably duplicates. Not all of these cultures have been adequately studied, and it may be that the more virulent actual pathogen was overlooked. The cultures reported upon in this paper are some of the isolates of which more study has been made, and that showed possibilities of being the cause of the melon disease.

Of the plant parts cultured, surface-sterilization affected only the seeds. It was found that non-treated seed yielded possible pathogenic bacteria, while the treated seed yielded no bacterial or fungal colonies at all (Fig. 7).

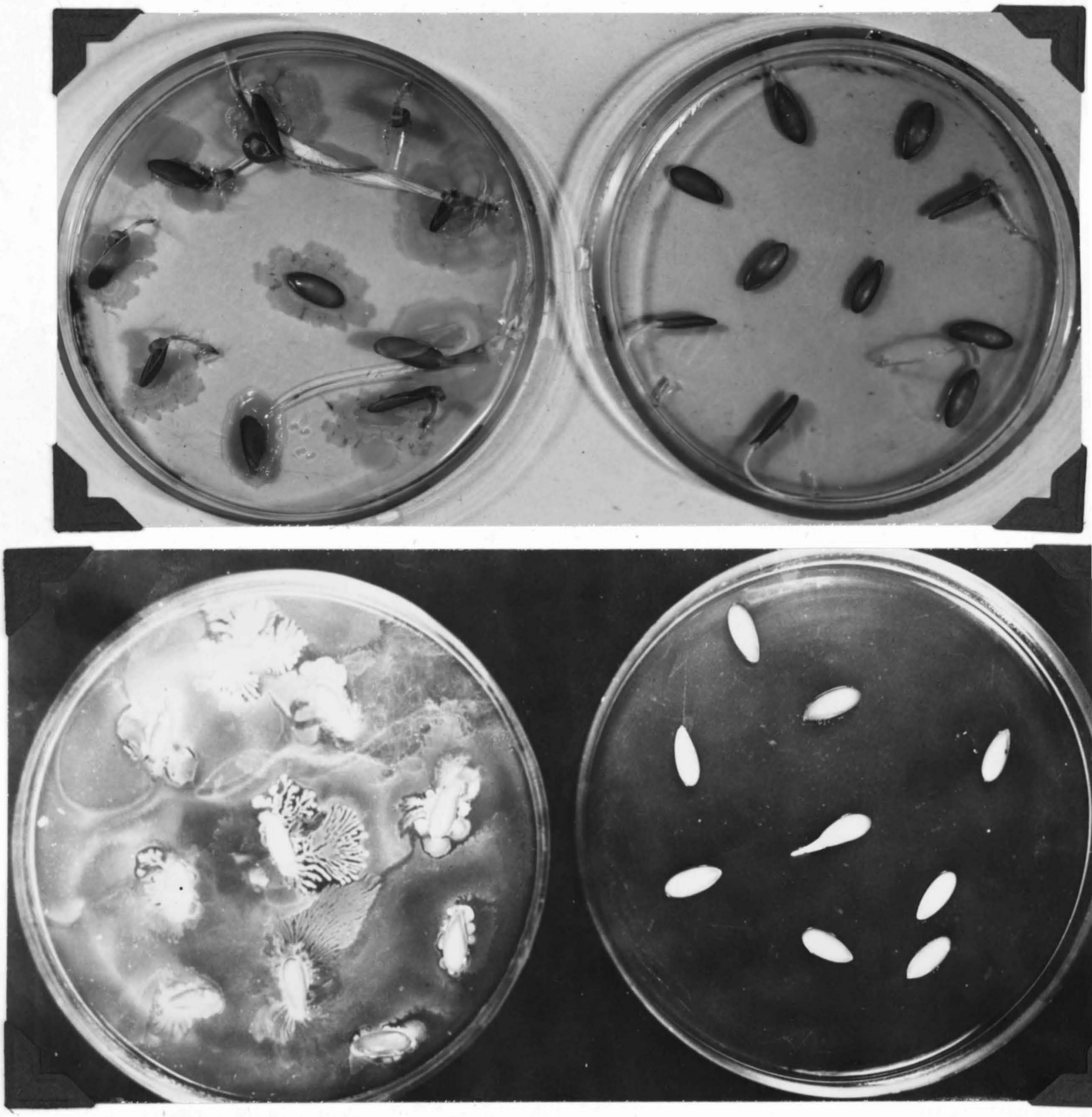

Figure 7

Upper left: Honey Dew melon seeds not treated
right: Honey Dew seeds treated with 1:1000 $HgCl_2$

Lower left: Cantaloupe seeds not treated
right: Cantaloupe seeds treated with 1:1000 $HgCl_2$

## PATHOGENICITY TESTS

Experiments to determine the pathogenicity of the bacteria were carried on in the laboratory, greenhouse, and out-of-doors. In the laboratory the work consisted of the rotting action of the bacteria upon certain vegetables: carrot root, cucumber, bell pepper, potato tuber, and ripe tomatoes. These vegetables were first dipped into 1:1000 $HgCl_2$, then sterile distilled water. They were then cut into slices which were placed in sterile covered Petri dishes having a moist cotton mat on the bottom. The slices were punctured several times with a flamed dissecting needle and inoculated by pouring several centimeters of a 24-hour bacterial suspension upon the slice of vegetable.

The results are shown in Table I, and all are in relation to a control set of vegetables. None of the isolates from cantaloupe plants agreed perfectly with the two standard cultures. This type of test was performed frequently, and the results were usually variable to doubtful.

In the greenhouse and out-of-doors, pots were planted with treated cantaloupe seeds, some from Ferry-Morse, variety Hales Best, and some from the Salt River Valley, variety Imperial 45. These seeds were treated either with Spergon, or dipped in 1:1000 mercuric chloride. This

TABLE I

Results of Vegetable Inoculation

| Culture | Vegetable | | | | |
|---|---|---|---|---|---|
| | Carrot | Cucumber | Bell Pepper | Potato | Tomato-ripe |
| B. carotovorus* | + | + | + | + - | + |
| B. aroideae (T)* | + | + | + | + | + |
| B. melonis* | + | | | + | |
| 1984 | + | + | + | ? | + |
| 5973 | + | + | + | + | + |
| T-Z | + | slight | + | - | + |
| Y | + | + | + | slight | - |
| CuY | - | + | slight | + | + |
| C | + | slight | + | - | + |

*Descriptions of B. carotovorus, B. aroideae and B. melonis in the above chart were taken from Massey.

resulted in healthy seedlings which was not the case when non-treated seeds of Imperial 45 were planted. When the first true leaf developed, the seedlings were inoculated either by (1) pricking the leaves and stems with a needle followed by spraying with a 24-hour bacterial suspension; or (2) spreading an inoculated oat mash about the plant. Out-of-doors a bell jar was placed over the inoculated plant for 24-48 hours; this was not done in the greenhouse where the humidity was greater.

Many of the results were doubtful and may have been confused or complicated by factors that will be mentioned later. In general, however, the two standard cultures, 1984 and 5973, showed good infection results; and the other isolates, with the exception of Y, were fairly constant in their ability to produce light infections on the seedlings. Some results of artificial inoculations are shown in Figures 9 and 10. The mash method tended to give a higher rate of infection, due to protection by the mash of the bacteria from too rapid drying. There was also a strong tendency for the seedling to outgrow the infection, as shown in Figure 10. This tendency was also noted in the field.

Seeds previously surface-sterilized were allowed to stand in a bacterial suspension for 10-30 minutes and then planted. With the exception of those contaminated with the culture T-Z, healthy seedlings were produced. The infection was light with the T-Z culture.


Figure 9. Results of artificially inoculated young cantaloupe plants, (i) possible infections. Oat mash method used.

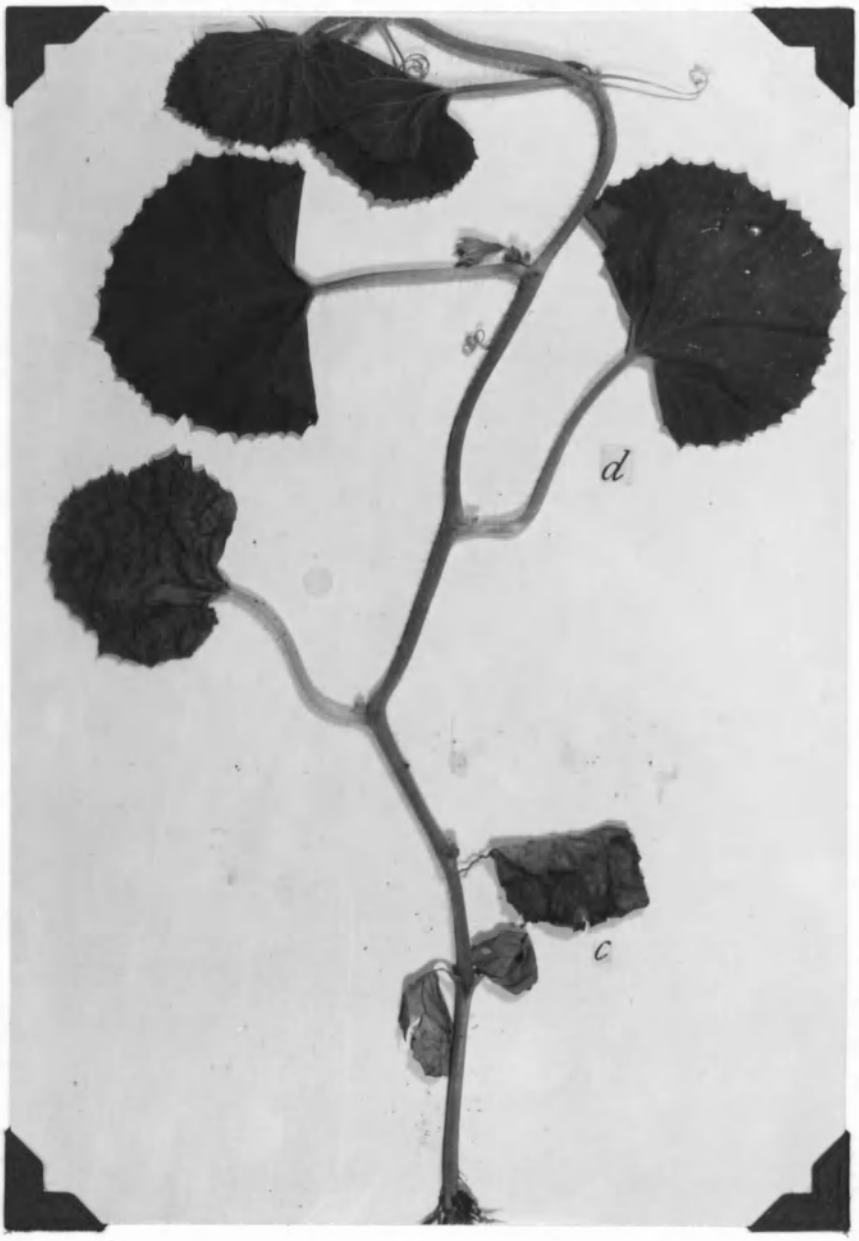


Figure 10. Artificially inoculated cantaloupe (CuY) seedling showing (c) infection, (d) tendency to outgrow infection. Spray method used.

## CULTURAL STUDIES

The bacterium causing this disease is believed to be a strain of E. aroideae of the soft-rot group. Cultural differentiation of members of this group has been based often on the fermentation of and gas production in several carbohydrate solutions. In these studies seven carbohydrates were used: l-xylose, dextrose, lactose, maltose, d-galactose, salicin, and dulcitol. The carbohydrate solutions were prepared by adding 2% of the organic carbon to either phenol-red broth base or to nutrient broth containing the indicator brom cresol purple. Both the closed arm fermentation tubes and simple inverted vials in ordinary test tubes were used to indicate the presence of gas. The solution was tubed, autoclaved and inoculated from an agar slant, 24 hours, or from a nutrient broth 24-hour culture.

The results were similar for the different indicators, tubes used and amount of initial inoculum. These results, Table II, show that none of the four isolates entirely agree with the two standard cultures, although CuY comes fairly close. One culture only was gas-producing. All of the isolates from cantaloupes seem to vary in their ability to ferment a given carbohydrate from time to time. One promising result was the lack of ability of some of the

TABLE II

Reactions of Cultures in Sugar Solutions

| Culture | Sugar Media | | | | | | |
|---|---|---|---|---|---|---|---|
| | l-Xylose | Dextrose | Lactose | Maltose | d-Galactose | Salicin | Dulcitol |
| E. carotovora* | A + G | A + G | A + G | | A + G | | |
| E. aroideae* | A | A | A | -- | A | A(Waldee) | |
| B. aroideae-T* | | A | A | B | | | |
| B. melonis-G* | | A | A | A B | | | |
| 1984 | A | A | A | --B | A | A | -- |
| 5973 | A | A | A | --B | A | A | -- |
| | | -B | B | - | B | - | |
| T-2 | A | A | A | B | A,sl | A,sl | -- |
| Y | A + G | A + G | A + G | A + G | A + G | A + G | A + G |
| CuY | A | A | A | A,sl | A | A | -- |
| | | | - | - | | - | |
| C | A | A | B | B | A | A | -- |

*Descriptions of E. carotovora and E. aroideae were taken from Bergey's Manual; B. aroideae from Townsend; B. melonis from Giddings.

A = acid; G = gas; B = alkaline reaction

bacteria to ferment maltose.

Other media used to determine certain biochemical characteristics included nitrate agar or broth, tryptone broth, starch agar, litmus milk and MRVP broth. These media were made up and the tests performed as specified in the Manual of the Society of American Bacteriologists(7). The same method of inoculation was used as for the carbohydrates.

Results are listed in Table III. As indicated, the results for indole production were regularly negative. The reduction of nitrates to nitrites and the hydrolysis of starch with a few exceptions were constant. The litmus milk was in all cases reduced, but later actions differed. The MRVP medium tests were the most variable. This acetyl methyl carbinol production as well as acid varied within a given culture to some extent.

TABLE III

Results of Culture Inoculation

| Culture | Culture media | | | | |
|---|---|---|---|---|---|
| | Indole | Litmus Milk | Methyl Red | Nitrite | Starch | Voges-Proskauer |
| E. carotovora* | - | Coag., acid, whey, reduced, sl. pep. | + | + | - | - |
| E. aroideae* | - | Reduced | - | + | slight | + |
| B. aroideae* | - | Acid, curd, whey, bleached | | + | | |
| B. melonis* | 10-20 da. | | | | | |
| | + | | | + | | |
| 1984 | - | Reduction, curd | + sl | + | - | + |
| 5973 | - | Reduction, curd | + | + | - | - |
| T-Z | - | Reduction | - | + | - | + - |
| Y | - | | + | + - | - | - |
| CuY | - | Reduction | + sl | + - | - | + sl |
| C | - | Reduction | - | + - | sl - | - |

*Descriptions of E. caratovora and E. aroideae were taken from Bergey's Manual; B. aroideae from Townsend; B. melonis from Giddings.

## PHYSICAL STUDIES

The morphological and physical characters of these bacteria were studied using nutrient broth, potato-dextrose-agar slants and plates and gelatin.

The size, form, and arrangement of the bacteria were studied from slides made from a drop of a 24-hour nutrient broth culture and stained with aqueous safranin. Their motility was observed in a hanging-drop slide. Attempts were made to stain the flagella, but thus far they have not been successful. The results are listed in Table IV. The bacteria used in these studies are all rods with rounded ends. All are motile and Gram negative but their size varies somewhat.

The agar-plate colonies made by pouring dilution plates were observed after 24 hours. The properties of both surface and deep colonies were similar for the six bacterial cultures. These results are also listed in Table IV, which shows the surface colonies to be circular, entire margin, elevation convex, white, glistening and amorphous to granular in internal structure. The deep colonies were spindle-shaped.

The growth on agar slants, Table V, differed in rate and in exact color, which ranged from white to gray-white,

TABLE IV

| Cultures | Morphological characters | | | | |
|---|---|---|---|---|---|
| | Size | Ends | Arrangement | Motility | Flagella |
| E. carotovora* | 1.5-5 x 0.7-0.8 | | Single chains | + | Peritrichous |
| E. aroideae* | 2 - 3 x 0.5 | Rounded | Single pair four chains | + | Peritrichous |
| B. aroideae* | 2 - 3 x 0.5 | Rounded | Single chains | + | 2-8 Peritrichic |
| B. melonis* | 1-1.7 x 0.6-0.9 | | | + | 4-6 Peritrichic |
| 1984 | 1.4-2.8 x 0.7 | Rounded | Single pairs | + | |
| 5973 | 1.2-3.7 x 0.6 | Rounded | Single pairs | + | |
| T-2 | 1.2-2.8 x 0.8 | Rounded | Single pairs chains | + | |
| Y | 1.6-4.8 x 0.8 | Rounded | Single clumps | + | |
| CuY | 1.7-4.2 x 0.6 | Rounded | Single pairs | + | |
| C | 1.0-2.8 x 0.6 | Rounded | Single pairs chains | + | |

*Descriptions of E. carotovora and E. aroideae were taken from

| Plate surface colonies | | | | | | Deep colonies |
|---|---|---|---|---|---|---|
| Form | Margin | Elevation | Color | Luster | Internal structure | Form |
| Circular | Entire | Convex | Grayish white | Glistening | | |
| Circular amoeboid | Sharp | | White | Glistening | Granular | |
| Round radiating | | | White | Opalescent shiny | Granular | Spindle |
| Round amoeboid | Entire | Slight convex | | Opalescent glistening | Finely granular | Spindle elliptical |
| Circular | Entire | Convex | White | Opalescent glistening | Homogeneous, finely granular | Spindle |
| Circular | Entire | Convex | White | Glistening | Homogranular | Spindle |
| Circular | Entire | Convex | White | Opalescent glistening | Finely granular | Spindle elliptical |
| Circular | Entire | Convex | White | Glistening | Finely granular | Spindle |
| Circular amoeboid | Entire | Slight | White | Glistening | Homo. finely granular | Spindle, irregular |
| Circular | Entire | Convex | White | Glistening | Homogeneous | Spindle |

Bergey's Manual; B. aroideae from Townsend; B. melonis from Giddings.

to having a faint yellowish tinge. The type of gelatin liquefaction proved to be divided into two main types: i.e., stratiform and narrowly infundibuliform, as shown in Table V. Inoculation of nutrient broth, Table V, resulted in all cases in turbidity, two showing surface growth and all but one showing sediment at the end of 4 days.

The thermal death points of the six bacteria were determined only within five degrees Centigrade. The method was that of immersing a TDP tube containing 5 cc. of a 24-hour nutrient broth culture in a hot water bath, thermostatically controlled, for ten minutes. Then transfers of the broth suspension were made on agar slants, and the growth recorded at 24-48 hour intervals. The results are listed in Table V, and indicate that the bacteria are able to withstand rather high temperatures.

TABLE V

| Culture | Agar slant Form | Gelatin Rate | Gelatin Type | Broth Surface growth | Broth Sediment | Broth Turbidity | T.D.P. |
|---|---|---|---|---|---|---|---|
| E. carotovora* | | Slow-Rapid | Surface | | Flocculent white | + | 41-51°C. |
| E. aroideae* | | | Narrow infund.# | | | + | 50°C. |
| B. aroideae* | Irregular | Fast | Entire | | Deposit slow | + | |
| B. melonis* | Spreading | | Infund. | | Slight | + | |
| 1984 | Filiform | Fast | Strati-form | - | + | + | 41-46°C. |
| 5973 | Filiform | Fast | Strati-form | - | + | + | 46-51°C. |
| T-Z | Filiform | Fast | Strati-form | + | + | + | 46-51°C. |
| Y | Spreading | Fast | Surface | - | Flocculent white | + | over 51°C. |
| CuY | Filiform | Slow | Narrow infund. | - | - | + | over 51°C. |
| C | Filiform | Fast | Strati-form | + | + | + | over 51°C. |

*Descriptions of E. carotovora and E. aroideae were taken from Bergey's Manual; B. aroideae from Townsend; B. melonis from Giddings.
#Infundibuliform

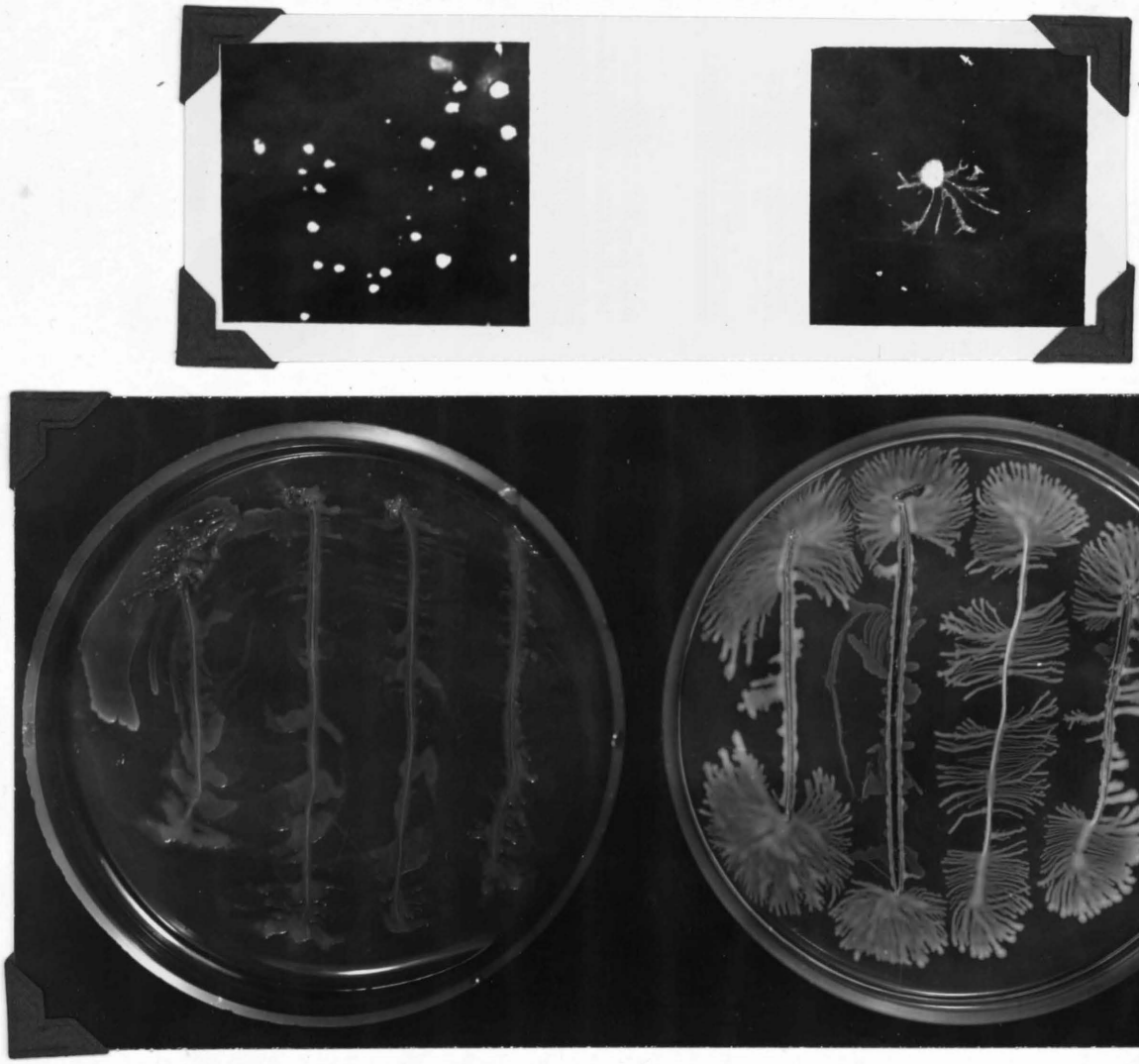

Figure 8
Upper left: Agar plate colonies of B. melonis 3 days growth at 25°C. Natural size
right: Agar plate colony of same age showing spreading type associated with abundant moisture.
Lower left: Agar streak of culture from rotted melon, 5 days.
right: Streak of culture from cantaloupe leaf, 5 days. Compare with upper right.

## DISCUSSION

The decreased incidence of infection with artificially inoculated plants as compared with the incidence of infection in the field is possibly due to one or more of several factors. It is difficult to reproduce field conditions and duplicate variable factors such as temperature, humidity, quantity of inoculum, pathogenecity of the culture, and action of insect vectors.

The first inoculation experiments were started in the summer of 1948. The lack of as thorough infections as compared to the amount in the field was thought to be influenced by the fairly constant high air temperatures which, although favorable to seedling development, may have exceeded the optimum for the rapid infection of tissues by the bacteria. However, the air temperatures are not accurate for the air layers immediately surrounding the plants; the atmosphere near the plants is subject to cooling resulting from transpiration of the foliage and evaporation of water from the soil. Thus the bacterial development might be slowed but not completely inhibited.

The humidity may have been too low, although an attempt was made to correct this by the use of bell-jars partly lined with moist filter paper. This was found to

be unsatisfactory for periods exceeding 24 hours, the increased heat and slow rate of evaporation causing the death of seedlings. These two factors, high air temperatures combined with a high rate of evaporation, may be largely responsible for the difficulty in obtaining a high rate of infection.

The quantity of inoculum sprayed on the seedlings is not believed to be a serious factor, for precautions were taken to inoculate with only very turbid broth cultures and to spray three times at 2-day intervals. Here the trouble may have been caused by too rapid evaporation of the broth before the bacteria had reached the inside of the host, there to be protected from drying.

The pathogenecity of the culture is a possible factor, especially in later inoculations. It is known that artificial culture of a pathogen may cause a variation, usually a decrease in its ability to attack and colonize a host.

Insects were a serious problem both in the field in 1948 and in the greenhouse. They are important in that they are probable vectors of the bacterium, both in the field and in the greenhouse, from inoculated to control plants. Sprays that did not harm the plant were used to control them, but they were not eliminated. Two insects causing greatest damage were an aphid and a leaf-miner. However, the variations in cultural and physical characters were often in accord with the contradictory results

experienced by other workers.

Considering all the possible infections and the similarities of cultures, allowing for certain fluctuations, it appears possible that these bacteria with the exception Y are strains of E. aroideae or at least members of the E. aroideae group (12) based upon their lack of gas production in carbohydrate solutions. Another indication of the variableness of the group is the large number of synonyms listed for E. aroideae in Bergey (1). The culture Y is, because of its gas production, a strain of E. carotovora.

Further work is needed to definitely determine the strain of the bacterium causing this disease of cantaloupes.

## SUMMARY

Several bacterial cultures have been isolated from cantaloupe plants from the Salt River Valley.

Some of these cultures were re-inoculated into cantaloupe seedlings with slight or doubtful infections.

Studies on the morphological and cultural characteristics were undertaken, as well as their rotting ability on a few vegetables.

The bacterium causing this disease is thought to belong to the soft-rot group, and may be a strain of either E. carotovora or E. melonis.

Further work is needed for its identification.

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