A STUDY OF BACILLUS AROIDEAE TOWNSEND, THE CAUSE OF A SOFT ROT OF TOMATO, AND B. CAROTOVORUS JONES.

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A STUDY OF BACILLUS AROIDEAE, TOWNSEND, THE CAUSE OF A SOFT ROT OF TOMATO, AND B. CAROTOVORUS JONES

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WITH THREE FIGURES IN THE TEXT

INTRODUCTION

In the summer of 1918, at Blacksburg, Virginia, there developed a considerable amount of a soft rot of tomatoes. This occurred in experimental plots which were designated to study the control of septoria leaf blight, and the soft rot of the fruit developed into an important factor. In describing these experiments Fromme (2) states: "Practically all of the unsoundness of the fruit was caused by bacterial soft rot, a disease which is exceedingly common and often very destructive in tomato fields in Virginia." Isolations from diseased fruits made by S. A. Wingard (15) proved a bacterium to be the causative agent. Its growth in pure culture resembled that of the group of bacteria which causes soft rots of plants but it could not be readily assigned to any of the described species of this group. There has been only casual mention of a bacterial soft rot of tomato in literature, and the distinguishing features of the organisms which might be responsible have not been as sharply defined as is desirable. It was decided, therefore, to undertake comparative studies of the organism in question together with some of the non-chromogenic soft rot forms.

Between the years 1898 and 1904 several species of soft rot bacteria were described. The literature of these is summarized by Harding and Morse (4) in their discussion of comparative cultural studies carried out by them. They describe here the situation of the soft rot forms up to 1909. In 1910 Giddings (2) described, under the name of *Baccillus melonis,* a soft rot bacterium found destructive to muskmelons and also capable of producing rot in some other plants.

Harding and Morse (4) seem to be the only ones who have made extensive comparative studies of the typical soft rot forms. Their studies included four named species (*Bacillus carotovorus* Jones,[2] *B. oleraceae* Harri-

[1] Paper No. 63 from the Department of Plant Pathology, Virginia Agricultural Experiment Station.

[2] In accordance to the classification recommended by the Committee of the Society of American Bacteriologists the names used here would be of the genus Erwinia, *Bacillus carotovorus* Jones, becomes *Erwinia carotovora* (Jones) comm. S. A. B., and *Bacillus aroideae* Townsend becomes *Erwinia aroidea* (Townsend) comm. S. A. B. These names are recognized but at this transition period it is found that it is clearer to use the old generic name Bacillus in this paper where synonomy is involved.

son, *B. omnivorus* Van Hall, and *B. aroideae* Towns.) and 39 strains isolated from various soft rot tissues. All of the 43 strains were alike in the 38 classificatory features studied except in their manner of fermenting the common sugars. *Bacillus carotovorus, B. oleraceae* and *B. omnivorus,* together with 30 of the unnamed strains composed one group on the basis that they produced acid and gas from dextrose, sucrose and lactose. *Bacillus aroideae* and three of the unnamed strains composed a second group which showed the characteristic of acid fermentation of these three sugars without gas formation. The six other unnamed strains were intermediate between the two groups and varied in the fermentation of these sugars, some producing acid and gas from one sugar and others from two. This shows a difference which it would seem is hardly sufficient basis for the establishment of distinct species. This is further strengthened by the fact that the gas fermenters are weak in their action. The amount of gas usually being just sufficient to recognize its presence in the fermentation tube. Harding and Morse question the advisability of considering *B. carotovorus* and *B. aroideae* as distinct species. However they recognize the fact that their behavior in pathogenicity may separate them more distinctly or show their close relationship.

COMPARATIVE STUDIES

Recognizing the results of the work of Harding and Morse as sifting the usually termed soft rot forms down at least to the two species, *B. carotovorus* Jones, and *B. aroideae* Town. and probably to one (the former), these two forms, along with the strain from tomato, were included in the study. *Bacillus melonis* was not included since a comparison of Gidding's description of this species and Townsend's description of *B. aroideae* fail to show clear specific differences (See table 3). Giddings does not seem to have compared his organism with *B. aroideae* and Erwin F. Smith who has studied the two together states (12, p. 240) "I think that *Bacillus aroideae* and *Bacillus melonis* are identical."

Our culture of *B. carotovorus* was secured from L. R. Jones and is a descendant of his original isolation maintained in his laboratory as No. 3a. The culture of *B. aroideae* was provided by Erwin F. Smith. It is not a descendant of Townsend's original isolation, but one isolated by Smith from calla and believed by him to be the true *B. aroideae.*

The investigations here reported are largely as to parasitism of the organisms in plants of the vegetable and floral groups and also their action towards various organic carbon compounds in pure culture. All inoculations and fermentations were carried out in duplicate and were repeated, with a few exceptions to be noted later. The experiments with the three organisms were carried in parallel and maintained under the same conditions.

PATHOGENICITY

The data here reported are as to inoculations carried on in the field, greenhouse and laboratory. All inoculations were by needle punctures into the uninjured healthy tissue. Potatoes, fruit, etc., in the laboratory were not sliced under aseptic conditions and placed in sterile dishes, but the whole structure was thoroughly cleaned and put into moist chambers and inoculated by puncturing with needle. Moist chambers were used which were large enough to accommodate material for two or more separate inoculations and one or two checks; the latter being punctured with sterile needle. In greenhouse and field, inoculations were made in the same manner into plants and plant parts *in situ*.

Methods of inoculation reported by previous investigators, in studying the soft-rot bacteria, have been of two types. The method which has been followed, as mentioned above, was also used by Jones (7) and is to-day largely used in investigations of wound parasites.

Townsend's method (13) of studying the action of *B. aroideae* was largely that of cutting slices of the plant tissue aseptically, placing in sterile petri dishes, dividing the slice into four parts, orienting them in the dish and finally two pieces in each dish "were inoculated with a 24 hour-old beef broth culture on the surface of the pieces and then stabbing through these drops—with a sterile needle." The objections to this method are twofold. The chances of autolysis of the plant tissue is greatly increased over that of the small wound caused by a needle puncture, hence the bacteria find simpler compounds at hand than those which occur naturally in the tissues. Secondly, the addition of a liquid culture to the wounded surface gives a chance for a saprophyte to appear parasitic since it is possible for the extra cellular enzymes to bring about hydrolysis of the compounds of the tissues. This is nicely illustrated in inoculation of the sweet potato with Rhizopus. Direct inoculations are rarely successful, but infection is more often obtained when the fungus is placed in contact with the wounded surface along with a broth in which the fungus has been growing.

It has been attempted to repeat as far as possible the inoculations reported by others, with these organisms under parallel conditions. Table 1 summarizes inoculations by previous workers, Jones, (7), Smith (12), and Townsend (13), and the results obtained by the author, with the two named organisms and the tomato strain.

The majority of inoculations made in the plants shown in table 1 gave the same results as have been before reported by other workers. As a whole, the negative and positive inoculations indicate a very close relationship between *B. carotovorus* and *B. aroideae,* and show the identity of the tomato strain with *B. aroideae.* They are sharply distinguished, however, by the reaction with certain hosts, notably cauliflower, kohl-rabi, and tobacco. The

TABLE 1.—*Results of inoculations of soft rot bacteria into vegetables, fruits and tobacco. O indicates no soft rot developed; + typical soft rot developed; blank spaces occur where no report has been made, or in author columns, inoculations not made.*

	B. carotovorus			*B. aroideae*		Tomato strain
	Jones	Smith	Author	Townsend	Author	Author
Apple, ripe	O		O	O	O	O
Banana, green			O		O	O
Banana, ripe	O		O	O	O	O
Beet root	O		O		+	+
Cabbage	+		+	+	+	+
Carrot, root	+	+	+	+	+	+
Cauliflower	O		O	+	+	+
Celery	+		+		+	+
Cucumber		+	+	+	+	+
Eggplant fruit	+		+	+	+	+
Kohl-rabi			O		+	+
Lettuce		+	+			
Muskmelon		+	+	+	+	+
Onion, youg leaf	+		O	O	O	O
Onion, mature	+		+	+	+	+
Parsnip	+		+	+	+	+
Pepper, fruit	+		+	+	+	+
Potato, sweet, root	O		O		+	+
Potato, white, tuber	O	+	+	+	+	+
Potato, white, stem	O		O		O	O
Radish	+		+	+	+	+
Salsify	+		+	+	+	+
Tomato, fruit	+		+	+	+	+
Tomato, young stem	O		O	O	O	O
Turnip, root	+		+	+	+	+
Tobacco, stem			O		+	
Tobacco, suckers			O		+	

type of rot produced by the two named organisms is very similar in all cases. Occasionally one finds a slight difference in the color and odor of the disorganized tissues. The results obtained with certain of the hosts are described in the following.

Cauliflower: The writer's inoculations with *B. carotovorus* in the cauliflower were negative agreeing with those of Jones while the inoculations with *B. aroideae* are positive and agree with those of Townsend. It was noticed, however, that the inoculations with *B. carotovorus* developed an initial appearance along a line of punctures as though rot were setting in. However, this initial appearance soon disappeared resulting in no disorganization of the tissue beyond the needle punctures. With *B. aroideae* and the tomato strain this initial appearance developed during the first 24 hours and was followed by rapid discoloration and general breaking down

of the tissues, typical of the soft rot. Harrison (5) reported a soft rot of the cauliflower by his *B. oleraceae,* and Harding and Morse (4), on the basis of their cultural studies, placed Harrison's bacillus in a group with *B. carotovorus.* Jones, as mentioned above, noticed a difference in the enzymatic activity of some soft rot forms. He found Harrison's bacillus to be more active than *B. carotovorus* but not as active as *B. aroideae.* It appears, therefore, that the enzymatic activity of *B. carotovorus* is not such as to enable it to carry out the disorganization of the cauliflower tissue.

Kohl-rabi: A number of inoculations were made into kohl-rabi "balls" both in moist chambers in the laboratory and in the "balls" growing in the

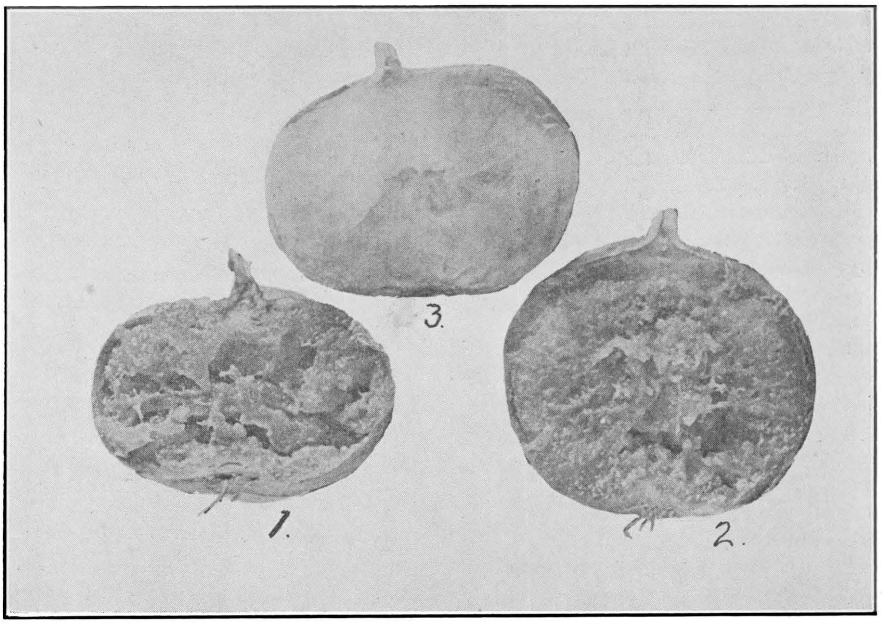

FIG. 1. Soft rot of Kohl-rabi balls induced by inoculation with (1) *Bacillus aroideae* and (2) the tomato strain; (3) Check, punctured with sterile needle.

greenhouse and in the field. The inoculations made with *B. aroideae* and the tomato strain developed rapidly into a mushy rot involving the parenchyma which is enveloped by the fibrous outer layer of the "ball." The rot does not become evident externally for a number of days. A foamy secretion is often noticed from the point of inoculation giving evidence of a considerable gas production. In a firm, succulent "ball" the internal break-down progresses rapidly and in about a week, occasionally in five days, the collapse of the outer wall of the "ball" becomes marked. In figure 1 are shown sections of kohl-rabi "balls" cut three days after inocula-

tion. No. 1 was inoculated with *B. aroideae;* No. 2 with the tomato strain; the upper figure, No. 3, is a check. In these figures are shown the general break-down of the tissues but no effect on the wall of the "ball." The odor of the decayed kohl-rabi is very disagreeable, of the nature of spoiled cabbage but stronger.

The inoculations made with *B. carotovorus* developed initial appearance as described in inoculations of cauliflower with this organism. The change did not go beyond this and even the initial appearance very soon disappeared. Checks which were produced by punctures with sterile needles did not show this change as was the case with the *B. carotovorus* inoculations; hence it could not be considered a case of traumatism.

Onions: Inoculations into mature onion bulbs developed rot in the case of all three organisms. However, the development of the rot by the *B. aroideae* and the tomato strain was slow. Townsend also found rot development in this host to be slow. The inoculations made by *B. carotovorus* developed into a more rapid rot and involved a larger area of the bulb. Only those bulb scales which were punctured with the inoculating needle were affected. The scales beyond the needle puncture become infected rarely and then apparently only through wounds caused by other agencies. Inasmuch as the organism is a wound parasite, this is exactly what might be expected since the bulb of course consists largely of succulent leaf bases. Inoculations into young onions were negative in both cases. Jones reports successful inoculations into the leaf. From his decsription, however, it would seem that the inoculations were made into the leaves of the sprouts from mature onions which were in moist chambers. The young leaves used in inoculations made here were those on young plants growing in soil in the greenhouse.

From this the conclusions to be drawn agree with the accepted facts to be found in the literature of today. Bacterial rot of the onion would not become troublesome in the field until late in the growing season when the bulb is maturing, its most serious damage more often developing in storage or transit and *B. carotovorus* being more actively responsible.

Sweet potato: Jones reports inoculations of *B. carotovorus* into sweet potatoes as unsuccessful and my tests with this organism were also negative. In the first experiments with the sweet potato *B. aroideae* and the tomato strain also proved non-pathogenic. All of these first inoculations were made into roots bought on the market. Later inoculations were made into roots freshly dug from the ground. In these *B. carotovorus* proved non-pathogenic while *B. aroideae* and the tomato strain developed a soft rot involving a small area of the root. The decay caused by *B. aroideae* was not extensive and it is not believed that it would develop a serious soft rot of the sweet potato.

White potato: Inoculations made into the tuber of the white potato from the market or from local storage did not develop pathogenically for *B. carotovorus* but did develop pathogenically in the case of inoculations with *B. aroideae* and the tomato strain. In the latter the decay was rapid and discoloration of the tissues was very marked, being similar to that produced by the blackleg organism. Later inoculations with *B. carotovorus* and *B. aroideae* into tubers recently dug developed into a rot in all cases the same results have been repeated by the writer several times. In these freshly dug tubers the rot by *B. carotovorus* is not essentially different from that produced by *B. aroideae.*

Tobacco: Johnson (6) observed a decay of the tobacco stems in the field which resulted in the development of a hollow stalk. He gives brief mention of the trouble and states that he isolated a bacterium from the diseased tissue with which he was able to reproduce the disease in other plants. He considered the organism one of the soft rot bacteria. More recently reports have come from Connecticut and Massachusetts of isolated cases of the same disease. In these latter cases it seems that no isolations of the organism were made and little or no attempt was made to establish its identity. The writer has made inoculations into tobacco stems and the stem of tobacco suckers with both *B. carotovorus* and *B. aroideae.* Inoculations made with *B. aroideae* developed a rapid browning of the pith followed by a general soft rotting and collapse resulting in a disappearance of the pith of the stem; consequently the "hollow stalk." These inoculations developed very markedly in 36 hours under greenhouse conditions (see figure 3). The inoculation made with *B. carotovorus* did not develop any discoloration or any disorganization of the tissues of the tobacco stalk.

The hollow stalk disease has been described as entering the base of the stalk or the tip and rapidly developing throughout the pith of the stalk and into the petiole and larger veins of the leaf. One plant becoming infected in the field through some wound may easily develop a source for spread of infection by various agencies, especially by the knife in topping. Clinton mentions an isolated occurrence of the disease in Connecticut and states that there are usually two or three plants together in the field. The writer has not found any report of any extensive development of hollow stalk. However, it is possible that the disease has been mistaken for the manifestations of other organisms. Specimens of diseased tobacco plants have been received from Virginia which were difficult to place and it seems probable now that some of these may have been cases of hollow stalk caused by *B. aroideae.* It is possible to confuse hollow stalk and tobacco wilt (*B. solanacearum*) on casual examination.

Striking differences in pathogenicity are also found among the floral plants, especially so in the calla lily and in the wild and cultivated iris. The results of inoculations into floral plants are presented in table 2.

TABLE 2.—*Results of inoculations of floral plants with soft rot bacteria.*

	B. carotovorus	*B. aroideae*	Tomato strain
Common Calla Lily, petiole *Zantedeschia aethiopica.* Var. *Minor*	O	+	+
Golden Yellow Calla, petiole *Zantedeschia Elliotians*	O	+	+
Elephant Ear, petiole *Caladium esculentum*	O	O	O
Garden Gladiolus, green leaf	O	O	O
Blue Flag *Iris versicolor* } Leaf	+	O	O
Wild Spring Iris *Iris verna* } Leaf	+	O	O
Hyacinth, Scape	+	+	+

As is noted in the table none of the organisms infected the elephants' ear nor the gladiolus, and all proved positive for the hyacinth. The action towards calla and iris is especially striking and is detailed as follows:

Calla lilies: Toward this host *B. aroideae* and the tomato strain are markedly pathogenic while *B. carotovorus* is not at all so. This is the host from which Townsend first isolated his organism. The usual illustration of the effect of this organism on cells shows the breaking down of the tissues over a limited area of the petiole. Such is not the most striking manifestation of the disease. The limited area rapidly enlarges resulting in a general destruction of the host as shown in figure 2. Leaf blade inoculation likewise results in a disorganization of the tissues. Inoculations from vigorous cultures into a good healthy petiole will show infection within 24 hours while those made into old petioles are slow in development. The writer has found this host to be the most satisfactory one for proving out *Bacillus aroideae.*

Iris: Inoculations into this host gave opposite results from the calla inoculations. *B. carotovorus* proved pathogenic while *B. aroideae* and the tomato strain did not. These inoculations were made mostly into the green leaf under very humid conditions. Infection is apparent as a general breaking down of the leaf tissues in a circular fashion around the needle puncture. Diseased areas on leaves which develop during periods of high humidity dry up and fall out when the humidity decreases, giving the appearance of shot hole, or if they occur on the edge of the leaf, resemble more the depredations of insects.

Observations for several years in a local garden where soft-rot of tomato has been destructive are of interest. In this garden soft rot of carrots, white potatoes, parsnips, cucumber, muskmelon and other plants was common while no rhizome or leaf rotting of iris has appeared. Irises taken from this garden proved by inoculations to be susceptible to *B. carotovorus.*

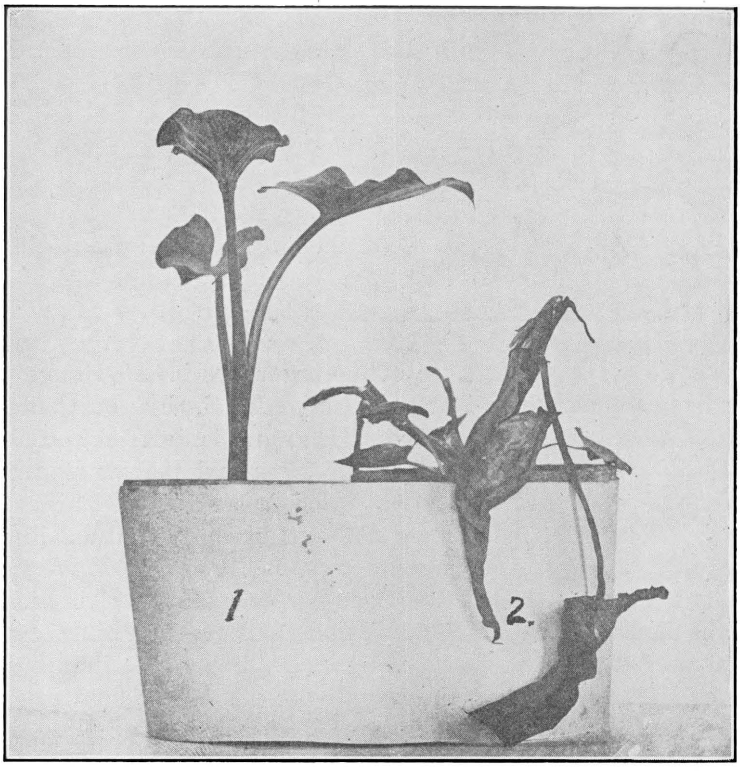

FIG. 2. Calla lily plants inoculated in petiole (1) with *B. carotovorus,* (2) with *B. aroideae.* No infection results from *B. cartovorus* inoculations.

Iris has been reported as subject to bacterial soft rot by several workers and it is not an uncommon disease. Specimens have been received from two localities in Virginia. Van Hall (14) described a disease of the young shoots caused by his *Bacillus omnivorus* (which according to the work of Harding and More mentioned at the outset, comes to *B. carotovorus*) on *Iris florentina* and *I. germanica.* Richardson (10) reports *B. carotovorus* as the cause of soft rot of iris in Canada. Shull (11), in a popular article, described the disease and measures of control.

Miss Lacy (9), in a short note, reports a soft rot of violet caused by *B. carotovorus.* So far our inoculations have all been negative. Difference

in varieties and conditions under which grown most likely is the explanation. The plants used in our work were cultivated forms that received no care. Miss Lacy's plants were from a garden where they were attended to and the growth was very likely much more succulent than was the case of the plants used in this experiment. Miss Lacy gives a very brief description of the organism she isolated and found to be parasitic and mentioned

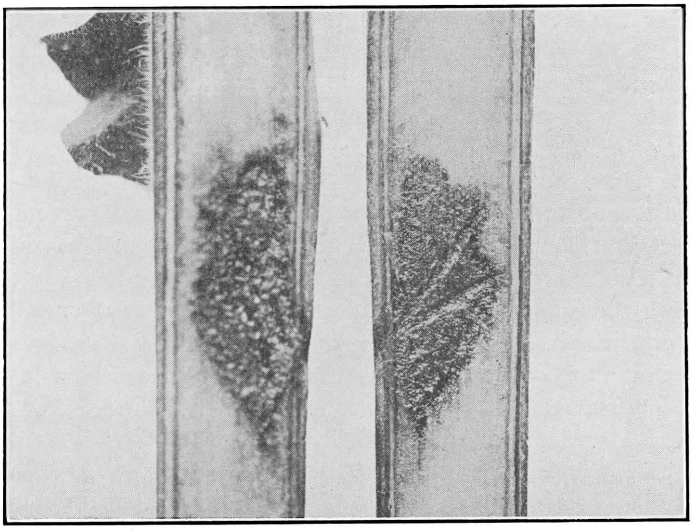

FIG. 3. Longitudinal section of tobacco stem 36 hours after inoculation with *B. aroideae*, showing disintegration of the pith.

that it corresponded favorably with *B. carotovorus* in culture in her laboratory but does not state that the pathogenicity of the latter organism was determined.

FERMENTATION

Cultural differentiation of *B. carotovorus* and *B. aroideae* has been mainly based upon the fact that the former produces gas where the latter does not. Bearing in mind the small amount of gas produced by *B. carotovorus* it makes the cultural differentiation rather uncertain.

The action of the organisms toward various organic carbon compounds were studied for the most part in solution. The cultures were grown in 150 cc. Erlenmeyer flasks, each containing 25 cc. of the culture solution. The solution was prepared by dissolving the total amount of organic carbon compound in four-fifths of the total amount of distilled water to be used. The concentrations of the organic compounds used are shown in table 4. After the solution was all dissolved 20 cc. portions were measured with

pipette into the flasks. These were plugged with cotton wool and sterilized in the autoclave under 15 pounds steam pressure for 15 minutes. Thus the carbon compounds were sterilized in the presence of the distilled water only. To each of these flasks was next added 5 cc. of sterile 2.5 per cent Bacto peptone solution. The transfer to the flasks was accomplished by means of sterile pipettes under guarded conditions to prevent contamination from the air. This made a peptone concentration of 0.5 per cent in each flask. After the sterile peptone solution had been added the flasks were incubated for three days to develop any contaminations. The percentage of contaminated flasks was very small.

The flasks were inoculated from 24-hour peptone broth cultures by putting into the flasks 2–3 drops of the culture from a pipette. The inoculated flasks were incubated at 25°–30° C. The hydrogen-ion concentration was determined at intervals to note the development of acidity and final pH. These determinations were made colorimetrically by comparing with standard buffer solutions containing the proper indicator. For detecting gas fermentation the agar shake method was used. The media was prepared in the same manner as described for the liquid cultures, *i.e.* by sterilizing the component parts separately. At first the oxygen relation in such cultures was questioned but experiments as shown in tables 5 and 6 cleared this point.

Table 3 summarizes the action of *B. carotovorus, B. aroideae* and *B. melonis,* as reported by Jones (7), Townsend, (13) and Giddings (3), respectively, and by Smith (12) for *B. carotovorus,* on some of the common organic compounds used in culture studies.

As shown in table 3 the three organisms are not distinguished on the basis of acid production, but *B. carotovorus* differs from the others in gas production. The amount of gas is often slight and this has not been considered a sufficient basis for separation by Harding and Morse (4).

The table shows how impossible it would be to separate Giddings's bacillus from *B. aroideae* in such cultures.

The fermentations here reported were carried out to determine the actions of *B. carotovorus* and *B. aroideae* toward a larger number of organic compounds in hope of showing stronger their relationship. This was carried out by observing the acid and gas fermentation of the following:

Alcohols

Monohydric—Ethyl, Butyl,
Polyhydric—Glycerol, Mannitol,
Aldehyde—Vanillin

TABLE 3.—*Gas and acid fermentation by B. carotovorus, B. aroideae, and B. melonis as gathered from literature.*

	B. carotovorus				*B. aroideae*		*B. melonis*	
	Gas		Acid		Gas	Acid	Gas	Acid
	LRJ	EFS	LRJ	EFS	Townsend		Giddings	
Dextrose	+	+	+	+	O	+	O	+
Lactose	+	+	+	+	O	+	O	+
Sucrose	+	+	+	+	O	+	O	+
Maltose					O	+	O	+*
Mannitol	+	+	+	+	O	+	O	+
Glycerol	O	O	+	+	O	+	O	+
Muscle sugar		+		+				
Milk	O	O	+	+	+	+**	+	+

* Giddings's results indicate acid development from maltose at 25° C. during first four days to be about pH 6.8, on 18th day about pH 7.4. Townsend's determinations were in twenty-weeks'-old cultures using litmus indicator.

** Reported by Smith (4). Townsend did not test the action of his organism on milk in fermentation tubes.

Monosaccharides

Pentoses—Arabinose, Xylose,
Methylpentose—Rhamnose,
Hexoses—Dextrose, Galactose, Levulose

Disaccharides—Saccharose, Maltose, Lactose,
Trisaccharides—Raffinose,
Polysaccharides—Starch, Dextrin,
Glucosides—Amygdalin, Esculin, Salicin, and Arbutin.

This selection presents a series of organic carbon compounds of varying complexities and was made with the hope of bringing out differences in the organisms through the selective action of their enzymes, that is, through the relationship between molecular configuration of the compounds and enzymatic action.

In the beginning it was realized that by observing only the acid and gas fermentations, the chances of finding differences in the action of the organism were limited. However, for practical differentiation of the organisms in culture a more detailed chemical study of the fermentation, as to the kind and amount of cleavage products, though interesting, and valuable, would not necessarily aid in readily distinguishing the organisms in cultures. It is hoped later to go into the study of these cleavage products which should help to explain points that come to mind in this study, and by chance throw

some light on the cause of resistance and susceptibility in plants to these bacteria.

The results of the fermentation of the compounds listed above, exclusive of the glucosides, are shown in Table 4. The hydrolysis of the glucosides is given in table 5. The relationship of the action on the mono-, di- and trisaccharides (exclusive of maltose) is the same as that shown by previous studies in the fermentation of the usual laboratory sugars. That is, *B. carotovorus* produces acid and gas, while *B. aroideae* and the tomato strain develop acid fermentation only.

The action towards maltose is contrary to previous reports. The writer found a slight rise in the H-ion concentration during the first 48 hours, but the action soon reverses. The figures in parenthesis opposite maltose is the pH. reading at the end of 24 hours. The most feasible explanation of this small acid development is that there seemed to be a trace, not readily detected, of inverted maltose in the culture. This was fermented with development of slight acidity and when used up, maltose proving unfavorable, the protein molecules are attacked to satisfy the carbon as well as the nitrogen requirements in the metabolism of the bacteria. The excess nitrogen being eliminated in the form of ammonia would increase the hydroxyl concentration. An analogous action towards the polysaccharides was found, though no initial rise in the pH.

Bio-chemical studies of the enzymes have shown that *emulsin* is commonly responsible for the hydrolysis of glucosides of the beta type, while the alpha-glucosides are hydrolized by the enzyme *maltase*. The beta-glucosides, amygdalin, aesculin, salicin, and arbutin were hydrolized by both organisms which indicates the presence of the emulsin complex in the cultures of these organisms. Maltose, glucose alpha-glucoside, is not hydrolized by these bacteria which indicates the absence of *maltase*. Demonstrating the presence of emulsin helps in further interpretation of table 4. Lactose, a glucose beta-galactoside, is more actively hydrolized by lactase. Some preparation of the emulsin complex from almonds has been found capable of hydrolizing lactose. The tri-saccharide, raffinose, is hydrolized by both emulsin and invertase; emulsin converting it to sucrose and galactose and invertase to fructose and melibiose. Melibiose (glucose beta-galactoside) is slowly acted upon by emulsin. Finding evidence of the presence of emulsin and invertase in cultures of these bacteria give a clearer understanding of the action shown towards the di- and tri-saccharides of table 4. Through the enzymatic action these compounds (exclusive of maltose) are hydrolized to the simpler monosaccharides and these are in turn fermented forming acid on the one hand and acid and gas on the other. It is in the fermentation of the monosaccharides that a careful bio-chemical study would be of interest and likely instructive as to the physiology of these closely related forms.

TABLE 4.—*Acid and gas fermentations by B. carotovorus and B. aroideae.*

		B. carotovorus			*B. aroideae**		
		H-ion concentration		Gas	H-ion concentration		Gas
		At start	4-day culture	7 days	At start	4-day culture	7 days
Alcohols and Aldehyde							
Ethyl	4.0 %	6.8	5.2	—	6.8	7.4	—
Butyl	0.5 %	6.8	7.0	—	6.8	7.2	—
Glycerol	6.0 %	6.8	6.8	—	6.8	7.0	—
Mannitol	1.0 %	6.8	5.0	+	6.8	5.0	—
Vanillin	0.02%	6.6	6.2	—	6.6	6.4	—
Monosaccharides							
Arabinose	1.0 %	7.2	5.6	+	7.2	5.6	—
Xylose	1.0 %	6.6	5.6	+	6.6	5.6	—
Rhamnose	1.0 %	7.0	5.2	+	7.0	5.4	—
Dextrose	1.0 %	6.6	5.0	+	6.6	4.4	—
Galactose	1.0 %	6.2	5.2	+	6.2	5.4	—
Levulose	1.0 %	6.6	5.2	+	6.6	5.4	—
Di- and Tri-Saccharides							
Sucrose	1%	6.8	5.0	+	6.8	5.0	—
Maltose	1%	6.8(6.2)	7.8	—	6.8(6.4)	7.2	—
Lactose	1%	6.4	5.2	+	6.4	5.4	—
Raffinose	1%	7.2	5.4	+	7.2	5.2	—
Polysaccharides							
Starch	1%	7.2	7.6	—	7.2	8.0	—
Inulin	1%	7.2	8.0	—	7.2	8.2	—

* The tomato strain agreed with the *B. aroideae* throughout.

The action on ethyl alcohol is of interest as it gives an additional means of cultural differentiation. The acid development from ethyl alcohol is very marked with *B. carotovorus* while it is entirely absent in cultures of *B. aroideae*. Growth in peptone broth containing ethyl alcohol is very striking. *B. carotovorus* produces a heavy pelicle and an abundant growth, while *B. aroideae* produces a very slight growth. This is well illustrated by Smith (12, fig. 184). The writer is not aware of acid fermentation of alcohol by *B. carotovorus* being previously recorded. By use of an alcohol-peptone, Brom-cresol-purple agar the two forms are readily separated as *B. carotovorus* quickly develops acid which changes the indicator from purple to yellow. *B. aroideae* grows on this agar, but there is no change in the color of the indicator.

The method of preparing the alcohol medium is as follows: The agar base contains 1 per cent peptone, 1.8 per cent agar shreds, and Brom-cresol-purple in distilled water. This is dissolved, tubed (8 cc. per tube) and sterilized. Just before use the agar is melted, cooled to 45° C. and 0.4 cc. of 95 per cent ethyl alcohol is added. The tube is rolled to mix and is quickly cooled as a slant. This can be held to prove its sterility if desired. The concentration of alcohol is 5 per cent. Ten per cent alcoholic concentration is not prohibitive to *B. carotovorus,* but seems to be to *B. aroideae* though the tolerance of the forms to alcohol has not received the writer's attention.

Stroke culture on agar of this type reveals acid development very prettily. For isolating *B. carotovorus* from rotted tissue it is of course essential that the plates be poured when the agar is first made on account of the volatility of the alcohol. On these plates *B. carotovorus* is readily isolated by fishing the acid developing colonies. If the rot is due to *B. aroideae* fishing from amoeboid non-acid colonies will assist in isolation.

The writer has also studied authentic cultures of *B. phytophthorus* and *B. atrosepticus* on alcohol-agar and both show no acid or gas production. They disagree with *B. carotovorus* in this respect although they are like it in a number of other respects according to facts gathered from literature and unpublished studies of the writer.

OXYGEN REQUIREMENTS

The growth of the soft rot forms in the closed arm of Smith fermentation tubes brought to mind the question of oxygen requirements. This was tested out in the presence of dextrose only. Two experiments were set up: The pH development was studied, first, in flasks and tubes presenting different surface areas exposed to the air of the vessel and second, in tubes with sterile vaseline covering the surface of the medium.

Experiment No. 1. pH development in dextrose peptone broth under varying surface area exposures. A dextrose peptone broth (peptone 0.5 per cent, dextrose 1 per cent) was prepared in bulk and then distributed in 25 cc. quantities in small (150 cc.) Erl. flasks 6.5 cm. in diameter at the surface of the liquid and large test tubes of 2.5 cm. diameter. After sterilization six of each were inoculated and each day for three days two cultures of each bacterium were used to determine the pH. Just prior to making pH determination in a culture the broth was thoroughly mixed and then determinations were made colorimetrically. The results are given in table 5.

Bacterium angulatum (Fromme & Murray) and *Bacterium tabacum* (Wolf & Foster) are included to show more strikingly the effect of surface area exposed on H-ion development. The tobacco bacteria are strict aerobes;

they do not grow in closed arm of Smith fermentation tube. This demonstrates the facultative nature of the soft rot forms. The availability of free oxygen is not a factor in the fermentation of the carbohydrates by them. The case is different with the tobacco leaf spot organisms. Also it is demonstrated clearly that it is necessary to consider the surface area of the culture in studies of H-ion development unless preliminary experiments show the organism to be a facultative form.

TABLE 5.—*Development of H-ion concentration in dextrose broth with varying surface area exposure.*

Bacteria studied	24-hour culture		48-hour culture		72-hour culture	
	Square centimeters in exposed surface					
	44.18	5.03	44.18	5.03	44.18	5.03
	pH	pH	pH	pH	pH	pH
B. carotovorus	5.0	5.0	5.0	4.8	4.8	4.8
B. phytophorus	5.2	5.2	5.0	5.0	5.8	4.8
B. aroideae	5.0	5.0	5.0	4.8	4.8	4.8
Bact. tabacum	5.2	7.0	5.0	6.4	4.8	6.6
Bact. angulatum	5.6	7.0	5.0	5.4	4.8	5.0
Control	6.6	6.6	6.6	6.6	6.6	6.6

Experiment No. 2. The pH development from dextrose in culture tubes with sterile vaseline covering the medium (Brown's (1) vaseline tube). The medium used here was the same as in the previous experiment; 10 cc. were placed in test tubes 1.5 cm. in diameter and then sterilized in the autoclave. As soon as the autoclave could be opened the tubes were taken out and about 1 cc. of melted sterile white vaseline was placed in each tube. In this way the chances of oxygen being absorbed were slight, if any. After cooling these were inoculated from agar cultures. The inoculation was made direct into the medium by holding the tube at about 45° angle and gently melting the edge of the vaseline which slips, thereby exposing the medium. After inoculation the vaseline plug is melted by holding the tube in the flame, tube placed upright, and the vaseline solidifies over the surface of the medium. That this vaseline seal is effective is shown by the fact that 9 tubes of broth prepared in this way, but not inoculated, standing for eleven months in the open laboratory, with only the protection of the cotton plug, show less than 1 mm. shrinkage.

The results of these anaerobic cultures are shown in table 6.

SUMMARY

A bacterial soft rot of tomato is here shown to be caused by *Bacillus aroideae* Townsend.

TABLE 6.—*Development of H-ion concentration and gas in vaseline sealed tubes in presence of dextrose.*

	pH	5-day-old culture	Gas
B. carotovorus	4.8		+
B. aroideae	4.8		O
B. phytophthorus	4.8		+
Bact. tabacum	6.6	No growth	O
Bact. angulatum	6.6		O
Control	6.6		O

Comparative studies of *B. aroideae* and *B. carotovorus* Jones, indicate the close relationship of these two forms, but they may be readily differentiated by either laboratory cultures, pathogenicity, or, better, by a combination of the two.

Bacillus carotovorus and *Bacillus aroideae,* though closely related, should be maintained as separate species.

The following scheme summarizes the differentiation of the two organisms:

I CULTURAL AND FERMENTATION CHARACTERS

	B. carotovorus	*B. aroideae*
Agar colonies in thinly sown plates	round, entire	amoeboid
Fermentation of: dextrose, lactose, galactose, saccharose, mannitol, etc.	acid and gas	acid without gas
Action in ethyl alcohol media	acid without gas, heavy pelicle and abundant growth.	no acid or gas, no pelicle and slight growth.

II PATHOGENESIS

Inoculation into:		
Calla	negative	positive
Iris	positive	negative
Kohl-rabi	negative	positive
Cauliflower	negative	positive

Studies of the oxygen requirements demonstrate the facultative nature of the soft rot forms and bring out the importance of stating the area of

the exposed surface of a broth in studies of H-ion concentration if the organism is not a facultative type.

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