A BACTERIAL LEAF SPOT OF HORSE-RADISH CAUSED BY BACTERIUM CAMPESTRE VAR. ARMORACIAE, N. VAR.¹

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INTRODUCTION

In 1926 several plants of horse-radish (Armoracia rusticana Gaertn., Mey. and Schreb.) growing in a greenhouse of the Department of Agriculture in Washington, D. C., developed a serious leaf-spot disease. These plants, with normal and vigorous leaf development, were grown from apparently healthy crowns planted some months before, and they had shown no sign of disease until late summer, when all the leaves were found thickly covered with spots. These particular plants were in the center of the house and partly shaded by cotton, papaya, and orange plants, all of which were free from disease. Some other horse-radish plants in the same house but on a bench where they received more light and air were so slightly infected that it required careful search to find the few spots that were present. Later the infection became more general on the bench plants, but never so heavy as on the partially shaded plants. The spots were full of bacteria, and isolation plates produced numerous colonies of a yellow bacterium with which characteristic lesions have been successfully reproduced on healthy horse-radish leaves. No fungi were found associated with the disease, and there was no trace of vascular infection.

In 1927 the same type of bacterium was isolated from leaf spots on horse-radish grown in Virginia (Arlington Experiment Farm), the District of Columbia (Terra Cotta), Connecticut, Iowa, and Missouri. None of the many naturally infected leaves sent in from these localities have shown any evidence of vascular infection.

In general appearance and growth the organism isolated from the horse-radish leaf spots closely resembled Bacterium campestre (Pam.) EFS., which so commonly infects many of the cruciferous plants. Further study proved that in morphological, cultural, and physiological characters it is practically identical with Bact. campestre and Bact. phaseoli EFS., but it is unlike these organisms in host reactions.

All the available literature on horse-radish diseases and on Bacterium campestre and Bact. phaseoli has been examined without any mention being found of a bacterial leaf-spot disease of horse-radish.

A root rot of horse-radish which is attributed to a bacillus (unnamed) has been reported from New Jersey several times since 1920 by Poole (22, 23, 24) and Martin (16, 17).² In England a very serious root rot of horse-radish was described in 1909 and attributed by the investigator (18) to Bacterium campestre. Sorauer (36) in 1899 reported a blackening and heart rot of horse-radish roots, but

¹ Received for publication Nov. 17, 1928; issued April, 1929.
² Reference is made by number (italic) to "Literature cited," p. 285.
did not determine the cause. Schleyer (30) in 1907 observed black points in cross sections of horse-radish roots, but he did not find or describe a causal organism. Smith (34) says that he has seen in the United States a disease of horse-radish which causes a crown rot and brown-stained vessels in the root. Faber (9) reports a vascular disease of stock (*Matthiola incana*) from which he isolated an organism morphologically and culturally like *Bact. campestrae*. Van Hall (10) describes a disease of summer stock (*M. annua*) which, according to Faber, is probably due to *Bact. campestrae*. Briosi and Pavarino (1) have described a disease of stock (*M. annua*) which caused some spotting of the leaves, but the more serious damage was due to vascular infection. *Bact. matthioliæ*, the organism described by Briosi and Pavarino as the cause of the disease, is culturally quite unlike *Bact. campestrae*.

From Chinese cabbages showing a characteristic vascular infection and consequent wilting and rot of the leaves, Brown and Harvey (2) isolated an organism which was similar to *Bacterium campestrae*. In addition to the vascular infection, "specking or spotting of the leaf blade may occur." On the Chinese cabbage the leaf spots, which apparently are the least serious phase of the disease, are in appearance not unlike those on horse-radish leaves. Brown and Harvey found that the organism isolated from the Chinese cabbage produced on the common cabbage a vascular infection and wilt similar to that caused by *Bacterium campestrae*.

**DESCRIPTION OF THE DISEASE**

The infected leaves bear numerous circular to angular spots, 1 to 4 mm. in diameter. (Pl. 1, A.) Young spots are uniformly translucent; when older they are more or less opaque, yellowish white to brown, or even black, with a dark, almost black border, which in turn is surrounded by a definite translucent zone. The lesion first appears as a tiny, translucent, pale-green point, visible only by transmitted light. Twelve to twenty-four hours later it is visible by reflected light as a small, dark-green spot. Young lesions are most noticeable on the lower side of the leaf. An infection that might escape casual observation from above would at once attract attention if seen from below. Fairly well isolated lesions attain a diameter of 4 mm., but the average size is 1 to 2 mm. (Pl. 2, H.) No bacterial ooze has been observed on the lesions. When 2 to 3 weeks old the spots have dull, dirty-white to brownish centers with a dark border, beyond which there is a halo of pale green.

By transmitted light the parchment-like center and the outer translucent zone are separated by the opaque, dark border. Variations, seemingly due to the degree of moisture, often occur; the outer translucent zone may be very narrow or even absent; occasional lesions are uniformly dark, others uniformly pale, and still others mottled with light and dark areas. With age the centers sometimes drop out. The vascular system of the leaf is not affected. Occasionally when a vein is included in a lesion it becomes dark, but the infection has never been observed to spread in the vein beyond the border of the lesion. (Pl. 1, C.) Leaf-margin infections occur, but the lesions remain small, and the infection does not extend into the veins. (Pl. 1, C.) Many leaf margins are entirely free from infec-
Bacterial Leaf Spot of Horse-radish

Inoculations on horse-radish with *Bacterium campestris armoraciae*: A and B, 20 days after inoculation, × 3/4; C, margin of leaf seven weeks after inoculation, × 2; D, right side of leaf inoculated on the upper surface, left side inoculated on the lower surface, × about 3/4; E, section of leaf five days after inoculation, × 250; F, stomatal infection four days old, × 800. (Photographed by James F. Brewer)
Bacterial Leaf Spot of Horse-radish

Plate 2

Bacterium campestrum armoraciae: A, Cauliflower two weeks after inoculation, slightly enlarged; B, cauliflower two weeks after inoculation, ×1; C, cabbage five weeks after inoculation with Bact. campestrum armoraciae, ×1; D, cabbage 17 days after inoculation with Bact. campestrum, ×1, for comparison with A, B, and C; E, Lima bean inoculated with Bact. phaseoli, ×2; F, Lima bean inoculated with Bact. campestrum armoraciae, ×2; G, marginal infection on horse-radish nine weeks after inoculation with Bact. campestrum, × about 4; H, typical Bact. campestrum armoraciae lesions on horse-radish 18 days after inoculation, × about 4; I, horse-radish eight weeks after inoculation with Bact. campestrum armoraciae, × 5. G, H, and I were taken by transmitted light. (Photographed by James F. Brewer)
tion. (Pl. 2, H, I.) Coalescing lesions may cause a general browning and drying of considerable areas of leaf surface, and heavily infected leaves become yellow and die prematurely. (Pl. 1, B.) After reaching the maximum size possible under the conditions, the lesions remain apparently unchanged for weeks, and unless heavily infected the leaf seems not particularly damaged by the parasite. In damp, warm weather new infections continue to appear both on the leaves originally infected and on those newly developed. (Pl. 1, A, B.) No trace of vascular infection has been found in any of the numerous leaves examined.

All parts of the lesions are full of bacteria, and from sections of fresh tissues the bacteria roll out in dense clouds. Fixed and stained sections show that in the early stages of the infection intercellular spaces and also cells that appear intact are filled with bacteria. Later the cell walls break down, and the cavity thus formed is filled with a dense mass of bacteria. A study of leaf sections shows that the infection is mostly, if not always, stomatal. (Pl. 1, E, F.)

ROOT DISEASES OF HORSE-RADISH

Previously and during the course of this investigation two root diseases of horse-radish have been encountered. One causes a soft rot and finally the entire destruction of all tissues except the tough fibers. In early stages of the disease the tissues are dull white to yellowish, spongy, light in weight; cells not separated; cell walls hyaline, later brownish; scanty to no starch in the cells; no odor other than the natural odor of the root. This sort of rot apparently starts at the crown and works downward. In some cases a wall of cork cells limits the rot and the roots partially recover and continue growth from side buds about a hollow or badly split-up crown. In microscopic examinations no organisms have been found except in the advanced stages of the disease. Isolations have given various sorts of organisms, none of them resembling Bacterium campestre.

The other type of root rot may be merely a phase of the one just described. Sometimes both kinds occur in the same root. In this disease the fibrovascular tissues are gray-brown to almost black. The tissues are firm; no organism has been found by microscopic examination, and isolations fail to give any organism resembling Bacterium campestre. This is probably the disease reported by Sorauer (56).

None of the plants with diseased roots, grown in pots in the greenhouse and in a small outdoor bed in Washington, have developed any bacterial leaf spots in the two years they have been under observation.

In cases occasionally seen where the horse-radish leaves are attacked by bacteria of the Bacterium campestre type and the roots by some sort of rot, one is forced to consider the possible relation between the disease on the leaf and on the root, the more so because a root rot of horse-radish has been attributed to Bacterium campestre by Middleton (18), and Pammel (20) found it causing a root rot of rutabagas, turnips, and rape. Pammel also mentions the dark fibrovascular bundles and states that soft-rot organisms follow the causal yellow bacteria. If either or both of the horse-radish root diseases observed by the writer should prove to be due to Bacterium campestre or a
closely related organism, the failure to isolate any organism from the petioles and midribs of leaves badly infected with the bacterial leaf-spot disease makes it appear that the infection does not reach the roots from the leaves by way of the vascular system.

**ISOLATION OF THE ORGANISM**

The bacteria are easily isolated from the leaf spots by the usual poured-plate method. The plates usually produced pure cultures of a yellow bacterium which when sprayed on healthy horse-radish leaves produced the typical lesions of the disease. Reisolations were made and infections again obtained.

Although no discoloration was observed in the vascular tissues, microscopic examinations of infected leaves were made, together with cultures from their veins, midribs, and petioles. The results of these examinations and cultures were negative.

**INOCULATIONS WITH THE HORSE-RADISH ORGANISM**

**HORSE-RADISH**

The disease is readily reproduced on horse-radish leaves by spraying them with a water suspension of the bacteria or by spreading the bacterial suspension over the leaf with a bit of absorbent cotton. The inoculated plants and uninoculated checks were usually kept in a moist, shaded case for 24 to 36 hours after inoculating. The first signs of infection are visible in 4 to 5 days as minute translucent spots. In 6 to 7 days, small, dark-green spots are found on the lower surfaces of the leaves. Still later the infection becomes visible on the upper sides of the leaves.

No infection resulted from prick inoculations made into the vascular tissues.

In spite of the fact that lesions are at first more distinct on the lower side, it appears that leaves inoculated on the upper surface become more heavily infected than those inoculated on the lower surface. To test this point, some leaves were inoculated on the upper side only and other leaves on the lower side only. In every case many more infections occurred on the leaves inoculated on the upper surface.

Another experiment was then made to determine whether this difference could be due to differences in plants, to the age of the leaf, or to the particular environment of certain leaves. On the selected leaves the right side or half (with the midrib as the dividing line) was inoculated on the upper surface. The other half (left side) was inoculated on the lower surface. These leaves were normal, well-developed blades having the two sides practically equal in area. By using a bit of absorbent cotton the bacterial suspension could be evenly spread over the desired area without contaminating other parts of the same leaf or any adjoining leaves. The plants were kept in a large case where there were no air currents or other disturbances to cause leaf movement and distribution of the infection. The results were even more striking than in the previous experiments. All the leaves (eight) developed numerous lesions on the right side of the midrib and comparatively few on the left side. By count there were seven to eight times more spots on the right sides than on the left sides. (Pl. 1, D.)
The horse-radish has numerous stomata on both sides of the leaves, and examination of the epidermis revealed no apparent differences in the size, structure, or number of the stomata of the upper and lower surfaces. Both sides of the leaf are easily wet with the bacterial suspension and apparently retain the moisture equally well.

CABBAGE AND CAULIFLOWER

Under very favorable conditions, cabbages and cauliflowers inoculated with the horse-radish bacteria developed leaf spots on areas from which the bloom had been wholly or partially removed. The leaves were inoculated by spraying heavily with bacteria in water or by applying the bacterial suspension to the leaf surface with a bit of cotton wool. From a number of such inoculations during the last two years leaf spots have been obtained on only about half of the plants and on only one leaf, or at most on two leaves, of the plant. Parallel inoculations with *Bacterium campestre* resulted in practically 100 per cent of the typical vascular infections characteristic of that organism.

The spots due to the horse-radish bacteria are usually small, pale gray-green, circular, sunken (pl. 2, A, B), and by transmitted light show a translucent halo. With age they become dark gray and dry. Water pores occasionally show signs of infection as tiny black points. In one experiment rather large marginal spots were produced. (Pl. 2, C.) These spots had definite dark borders unlike the indefinite margin of infections due to *Bacterium campestre*. The infection did not progress in the leaf tissue, and the veins in the lesion and surrounding tissue were not darkened.

Microscopic examinations show that the bacteria are usually not very abundant in the lesions on cabbage and cauliflower leaves. In some no bacteria could be found, and isolation of the organism was successful only after repeated trials. This scarcity of bacteria in the lesions suggests that in some cases the damage may be more or less due to physical or chemical causes. The reisolated organism from both cabbage and cauliflower was successfully tested for pathogenicity on horse-radish. In no case was there any discoloration of vascular tissue in the cabbage or cauliflower, and efforts to isolate the bacteria from vascular tissues adjoining leaf spots were not successful.

The bacteria reisolated from cabbage and from cauliflower lesions were again used to inoculate cabbage and cauliflower leaves, but the results did not indicate any increased pathogenicity from passage through these hosts.

In all these inoculation experiments, check inoculations were made on horse-radish leaves, which always developed numerous typical spots.

In addition to the spray inoculations, both cabbages and cauliflowers were heavily inoculated by pricking the bacteria into the vascular tissues of stems, petioles, and leaves. In one case each of cabbage and cauliflower, five months after inoculations were made by pricking the bacteria into stalks just above the petioles, a few dark streaks were found in the stalks near the inoculated areas. (Inoculation wounds still visible.) Adjacent leaves showed no signs of disease. Cultures were made, and from the cauliflower the yellow organism was recovered. Except for this slight evidence no vascular
infections were obtained on cabbages or cauliflowers when inoculated with the horse-radish bacteria.

Check plants of both cabbage and cauliflower of the same age and variety as those inoculated with the horse-radish bacteria, when inoculated with *Bacterium campestre* by pricking, developed the characteristic vascular infection of that organism.

**STOCK**

Two inoculation experiments with stock (*Matthiola* sp.), in which the bacteria were sprayed and rubbed on the leaves, gave no infections. In the third trial a few pale, irregularly shaped spots appeared. The tissues in the lesions were collapsed and very thin. Under a lens the veinlets showed as dark lines in the pale tissue. The typical yellow bacteria were isolated from the spots. In this third attempt bacteria had also been pricked into the stems. Two and a half months later one of the plants had yellow, sickly looking leaves. Examination showed dark vessels in the stem in the vicinity of the inoculation wounds. In a microscopic examination no bacteria were found, and isolation plates made from the tissues produced only four yellow colonies which in cultural tests proved to be like the organism used to inoculate the plant.

**LIMA BEAN**

One experiment with Lima beans under favorable conditions (checks were successfully infected with *Bacterium phaseoli*) gave no trace of infection. A second experiment under apparently similar conditions resulted in a number of spots on young leaves not yet fully developed. On the sixth day the spots were mere specks, dark and angular in the center of pale-yellow areas having indefinite margins. When 10 days old they were still small (one-fourth mm. and less), angular, dark reddish brown, and opaque (pl. 2, F); whereas the checks (parallel plants inoculated with *Bac. phaseoli*) had lesions 1 to 1½ mm., dull yellow brown, and very translucent (pl. 2, E).

**OTHER PLANTS**

Mustard, radish, kale, nasturtium, candytuft, wild cress, squash, poppy, cotton, navy bean, and cowpea were inoculated, but no infections were secured.

**INOCULATIONS WITH BACTERIUM CAMPESTRE**

**HORSE-RADISH**

When the leaves of a horse-radish plant are sprayed or rubbed with *Bacterium campestre* and kept very moist for several days lesions sometimes develop on one to three of the leaves. Evidently the bacteria can attack only leaves of a certain age. The lesions are few in comparison to those produced by the horse-radish bacteria and also slower (eight days) in developing. When first visible they are similar to lesions caused by the horse-radish bacteria; when older they may be entirely like the usual horse-radish spots, or they may be uniformly opaque, dark brown to black, sunken, alike on both sides of the leaf.

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1 Three strains of *Bacteria campestre* were used: A, Received in 1926 from Wisconsin, age unknown; B, a subculture resulting from infection of cabbage with A; C, isolated in 1927 from infected cabbage sent from Wisconsin. All 3 strains produced in cabbages and cauliflowers the characteristic vascular disease known as black rot.
and with no translucent border or only a trace of it. Leaf-margin infections were good in one case. (Pl. 2, G.) In most of the inoculation trials no infection occurred.

Check plants inoculated with the horse-radish organism never failed to give large numbers of lesions on all but the very youngest leaves. Leaves were inoculated by pricking \textit{Bacterium campestre} into the vascular tissues of midribs and petioles. No infection resulted. Smith (34) inoculated horse-radish with \textit{Bact. campestre} but obtained no infections.

\textbf{CABBAGE AND CAULIFLOWER}

Cabbage and cauliflower plants of the same age and variety as those inoculated with the horse-radish bacteria were inoculated with \textit{Bacterium campestre}, and the typical "black rot" vascular infection resulted. The inoculations were made by pricking the bacteria into the vascular tissues at the base of the petioles and on midribs and also by spraying the leaves with bacteria in water. In the spray inoculations the infection started in the water pores and spread into and darkened the veins. (Pl. 2, D.) The leaves gradually yellowed and fell off. No leaf spots occurred in these experiments unless the bloom had been removed from the leaves. In such cases leaf spots developed which looked quite like those produced by the horse-radish bacteria under similar severe inoculating conditions.

The waxy bloom on the leaves of these plants prevents wetting, and a fine spray, even if it remains on the leaf surface, may not be in direct contact with the epidermis and the stomatal cells. The large water pores on the leaf margin are usually the only places where the bacteria in water can gain entrance to the interior of the leaf. Russell (28) reports that no leaf spots are caused by spray inoculations of \textit{Bacterium campestre} unless the leaves are injured. Clayton (4) reports lesions due to \textit{Bact. campestre} occurring in the field directly through the lower leaf surfaces of cauliflower, Brussels sprouts, and cabbage. Samuel (29) describes nontypical \textit{Bact. campestre} lesions on cauliflower in the form of small black leaf spots. He considers this type of infection as probably due to unusual weather conditions.

\textbf{LIMA BEAN}

Leaves of Lima bean were inoculated with \textit{Bacterium campestre} in six different experiments. No infections were obtained. (These beans, Fordhook variety, were very susceptible to \textit{Bact. phaseoli}.)

\textbf{INOCULATIONS WITH BACTERIUM PHASEOLI}

\textit{Bacterium phaseoli}, the cause of a leaf spot of beans, is morphologically and culturally almost identical with \textit{Bact. campestre} and the horse-radish leaf-spot bacteria. In two different experiments horse-radish leaves were inoculated by spraying with \textit{Bact. phaseoli} suspended in water. The plants were kept in a moist case for three days. In both experiments fairly good infections were obtained. The lesions were first visible on the seventh day, later becoming quite definite, being in character like those produced by the horse-radish bacteria. They were somewhat slow in growth, however, and new

\footnote{Two cultures were used—one isolated in 1926, the other in 1927. Both were obtained from Miss Florence Hedges.}
infections did not occur as is the case when the infection is due to the horse-radish bacteria. One plant, kept unusually moist, 19 days after inoculation, had lesions 1 to 2½ mm. in diameter, but no new lesions. Lima beans and cowpeas inoculated at the same time with Bact. phaseoli, developed heavy infections first visible on the fourth day after inoculation.

In five different experiments, leaves of growing cabbages were inoculated with Bacterium phaseoli. Some of the plants were young, having 6 to 10 leaves; others were beginning to form heads. Bacterial suspensions were sprayed on the leaves, and bacteria direct from the cultures were spread over the leaves. The inoculated plants were kept moist for periods varying from three to seven days. Not a trace of infection resulted.

CULTURAL CHARACTERS OF THE HORSE-RADISH ORGANISM

Preliminary study had demonstrated that the bacteria causing leaf spot of horse-radish have characters similar to several others of the already rather large group of yellow plant pathogenes. For this reason, in practically all of the cultural work, parallel cultures of Bacterium campestre were made for comparison, and in a number of media the horse-radish organism was compared in parallel cultures of Bact. phaseoli, Bact. phaseoli sojense, Bact. malvacearum, Bact. cucurbitae, Bact. stewartii, and Bact. hyacinthi.

In the rather extensive list of publications relative to Bacterium campestre, only three papers have been found—Harding (11), Rüssel (28), and Smith (34)—which give detailed descriptions of the morphological and cultural characters of the organism. These investigators found that cultures of different origins and different ages often varied more or less in cultural characters and in virulence. Brown and Harvey (2) came to the same conclusion.

Dufrenoy and Szymanek (7) describe a cauliflower disease that occurs in France and has symptoms like those produced by Bacterium campestre and a causal organism similar to but not identical with Bact. campestre. Paine and Nirula (19) report a root rot of swedes (rutabagas) and turnips having general symptoms unlike those of Bact. campestre, but a causal organism similar to Bact. campestre. They also state that their organism corresponds exactly with the one isolated in France from cauliflower by Dufrenoy and Szymanek (1 to 4 flagella; faintly chromogenic; colonies on agar, white with yellow papilla; gelatin liquefied rapidly; nitrate reduced; diastatic action decided but moderate; distinct alkaline reaction from glucose sucrose, and lactose). These authors are inclined to view their organism as a saltant, a biological strain of Bact. campestre.

In the present study no variations other than the rough colonies (p. 277) have been observed in the several strains of the horse-radish bacteria, but these are all of recent isolation and may show variations after a longer period in artificial culture. In general, they grow more vigorously in culture than either Bacterium campestre or Bact. phaseoli.

1 Detailed descriptions of Bacterium campestre are given by Harding (11), Rüssel (28), and Smith (34). These publications may not be available to all workers. Other published descriptions of Bact. campestre are lacking in various ways, so it seems advisable to give a fairly detailed description of Bact. campestre var. armoraciae. This description is mostly in tabular form (see Table 1).

6 See footnote 3, page 274.
Unless otherwise stated, the cultures were grown at room temperature. Beef medium was made from fresh beef infusion and had a pH value of 7.0. Colors are according to Ridgway (27).

**BEEF-AGAR COLONIES**

Small white colonies are visible in two days. The color soon becomes pale yellow (massicot yellow) and later amber or wax yellow. They are round, flat, smooth, shining, translucent, with entire margins. Typical colonies are uniform in structure. Under the microscope they are finely granular, with a few coarse granules in the center. Colonies frequently show interior markings, as delicate striae, usually concentric, occasionally radial in arrangement, or a mottled, loose, almost lacy structure. Some colonies have a wide border or zone less dense than the center. The markings gradually disappear. Well-isolated colonies are 6 mm. in diameter when 10 days old. In texture the growth is smooth and soft. Buried colonies are oval to spindle shaped. When 2 to 3 weeks old there is a renewal of growth in the form of small circular colonies breaking through the original growth and also wedge-shaped growths at the margin. The new growth is lighter in color than the old and often exceeds the original colony in area. This renewal of growth is much more vigorous and extensive in the horse-radish organism than in Bacterium campestre. Both surface and buried colonies are usually somewhat brighter yellow and more transparent than colonies of Bact. campestre developed at the same time and under identical conditions.

**ROUGH COLONIES**

Rough-surfaced colonies appear occasionally in most of the isolation plates made directly from leaf spots, and sometimes every colony in a set of plates has the atypical structure. These colonies are smaller in size and brighter yellow than the normal colonies and have from one to several distinct papillae, or convolutions, on the elevated surface, which give them the appearance of miniature raspberries. They are roughly circular; margins irregularly lobed; growth thick, opaque, piled up in contrast to the flat, translucent, normal type of colony. If growing conditions are favorable, well-isolated rough colonies become smooth in 10 to 14 days, but the interior retains various irregular markings, these most often as radiating ridges. With age and drying the rough surface sometimes reappears. In texture the rough colonies are dense and firm, not viscid, but cohering so firmly that in making transfers the whole colony is picked up by the needle. On most culture media the growth is brighter yellow and denser in texture than growth from normal colonies. The surface is often dry and granular in appearance. On beef-agar slants the growth is firmly attached to or incorporated with the surface of the agar and is not easily transferred. Beef broth clouds less readily and has a tendency to form pseudozoogloeae, and milk cultures produce less tyrosine than the normal smooth growth.

Single, well-isolated rough colonies, when replated to test the permanency of the rough character, gave varied results. Sometimes all the colonies obtained from a single rough one would be rough, another would produce only smooth colonies, while others gave a mixture of rough and smooth colonies.
<table>
<thead>
<tr>
<th>Specifications</th>
<th>Bacterium campestrum armoraciae, n. var.</th>
<th>Bacterium campestrum (Pam.) EFS.</th>
<th>Bacterium phaseoli EFS.</th>
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<tbody>
<tr>
<td>Index number</td>
<td>211.222512</td>
<td>211.222512 (L. McC.)</td>
<td>211.222512 (F. H. (18).)</td>
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<tr>
<td>Horse-radish leaves; definite, distinct spots in leaf parenchyma; no wilting; no vascular infection.</td>
<td>Cabbage, cauliflower, and other crucifers; vascular infection, causing wilting and dropping of leaves.</td>
<td>Beans (bush and Lima); primarily a leaf-parenchyma infection. Vascular system sometimes infected. (F. H. (14) Burk. (5)).</td>
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<td>Host and general effect</td>
<td></td>
<td>Occasionally, and under conditions very favorable for the bacteria, a few leaf spots develop; these are similar to those produced by the horse-radish organism. (L. McC.)</td>
<td>Results of inoculation like those from inoculation, with Bacterium campestrum. (L. McC.)</td>
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<tr>
<td>Horse-radish.</td>
<td>Numerously definite spots develop in the leaf parenchyma; infection through stomata; little or no ooze from the lesions; lesions not shining when dry; infection continues to spread in the form of spots on the same leaf and to other leaves; no trace of vascular infection; no wilt.</td>
<td>Typical vascular infection, with consequent yellowing, wilting, and death of the leaves. If the waxy bloom is removed from the leaves, spray inoculations sometimes result in definite, small leaf spots. (L. McC.)</td>
<td>In five different experiments no trace of infection was secured on cabbage or cauliflower even under most favorable conditions. (L. McC.)</td>
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<td>Effect on inoculated horse-radish.</td>
<td>Under conditions very favorable for infection, spray inoculations sometimes produce leaf spots comparatively few in number, small in size, and unlike Bact. campestrum lesions. One out of many prick inoculations developed after several weeks a doubtful vascular infection.</td>
<td>Leaf spots result only when leaves are rubbed or wounded. (Russell (58)). Leaf spots occur under some natural conditions. (Clay. (4)).</td>
<td>No success in infecting cabbage with Bact. phaseoli. (EFS. (52), (53)).</td>
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<td>Cabbage and cauliflower.</td>
<td>Very small, opaque spots, quite unlike Bact. phaseoli lesions, are sometimes produced.</td>
<td>Repeated inoculations on bean failed to give infections. (EFS. (53)). In six different experiments no trace of infection. (L. McC.)</td>
<td>No infection obtained on cabbage. (Johnston*).</td>
</tr>
<tr>
<td>Effect on inoculated beans (bush and Lima).</td>
<td>A few, but definite, leaf spots and in one case a possible vascular infection.</td>
<td>Vascular infection. (Faber (9)).</td>
<td>Typical infections easily obtained. (L. McC.).</td>
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<td>Stock (Matthiola).</td>
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<td>Effect on inoculated turnip.</td>
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<td>Morphology.</td>
<td>On beef agar, single rods, 0.7 to 2 by 0.3 to 0.5 μm; some paired, no chains observed; many irregular forms. On potato or media with sugar, the average size is larger than on beef agar; long chains occur; forms are regular, 0.7 to 2.7 by 0.3 to 0.7 μm. Single polar flagellum. Distinct capsule.</td>
<td>In parallel cultures, like armoraciae. (L. McC.) Rod shaped, singly, in pairs, or fours; often irregular in form: 0.7 to 3 by 0.4 to 0.5 μm. (EFS. (54)). 1.87 to 3 by 0.37 μm. (Pam. (2)) 0.7 to 3 by 0.4 to 0.5 μm. (Doidge (6)). 1.0 to 2 by 0.4 to 0.6 μm. (Hard. (11)). Single polar. (EFS (54), Doidge (6)).</td>
<td>In parallel cultures, like armoraciae except for greater extremes in size; on beef, 0.5 to 2.7 by 0.5 to 0.6 μm; on potato and sugar media, 0.5 to 3 by 0.3 to 0.5 μm. (L. McC.) Rods, single, paired, and in chains. (EFS. (55), Wolf (57)). 1.3 to 2.0 by 0.6 to 0.7 μm. (Wolf (57)). 0.5 to 0.9 by 0.2 to 0.5 μm. (Rapp (52)).</td>
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</tbody>
</table>

Colonies on beef-agar plates.  White, changing to pale yellow, then deeper yellow; uniform structure, only occasionally ringed; translucent, becoming transparent; in parallel cultures, usually brighter and clearer yellow than *Bact. campestris*.  (Bacterial-agar used in all experiments by L. McC.)

Beef-agar stabs.  Surface growth seldom reaches wall of tube; a trace of growth at first in the upper part of the stab; white, chemical film over surface not covered by the bacterial growth.

Beef-agar slants.  Smooth, becoming slightly contoured and transparent; apricot color, deeper yellow than in plate; growth sometimes slightly viscid.

Beef bouillon.  White chemical film.  Clouds thinly in 24 hours; growth best at the top; if undisturbed, thin, ragged pellicles and rims form; sediment yellow, translucent, slightly viscid, rises in a spiral when rotated.

Whey agar.  Yellow color of growth less bright than on parallel cultures of *Bact. campestris*.

Beef gelatin.  Beef-infusion gelatin at 18° C. liquefaction begins promptly but requires 2 to 3 months to complete; liquid clear; sediment, bright yellow (apricot); no rim; no pellicle.  Beef-extract gelatin at 15° C.; liquefaction more rapid than the beef-infusion gelatin; liquid cloudy; sediment dirty, yellowish brown; thin rims and pellicles.

Distinct capsule.  (L. McC.)
No capsule.  (Dodge (6), Hard (11).)
No spores.  (Dodge (6).)
Gram-negative.  (Hard (11).)
Not acid-fast.  (L. McC.)

Both surface and buried colonies usually less yellow and less transparent than *armoraciae*; more likely to have ringed structure.  (L. McC.)
Pale yellow, deepening with age.  (EFS. (54).)

Like *armoraciae*.  (L. McC.)

Like *armoraciae* except less transparent and slightly less yellow.  (L. McC.)

White chemical film.  (L. McC.)
Like *armoraciae*.  (L. McC.)
No true pellicle or rim; yellow, granular precipitate.  (Hard (11).)

Strong yellow color; deeper yellow than with the horse-radish organism.  (L. McC.)
No liquefaction.  (Pam. (81).)
Liquefaction slow.  (EFS (3), Hard (11), Dodge (6).)
Liquid clear, copious precipitate.  (Hard (11).)

Single polar.  (EFS. (3), Dodge (6), Rapp (86), Wolf (37).)
Distinct capsule.  (L. McC.)
No capsules observed.  (EFS. (3), Rapp (86), Wolf (37).)
No spores observed.  (EFS. (3), Rapp (86), Wolf (37).)
Gram-negative.  (EFS. (3), F. H. (14), Rapp (86), Wolf (37).)
Not acid-fast.  (Rapp (86).)
PAL yellow, deepening to wax.  (Rapp (86).)
Pale to deeper yellow.  (EFS. (34).)
Often pale ringed.  (EFS. (35).)
Plainly ringed in 10 days.  (Rapp (86).)

Like *armoraciae*.  (L. McC.)
Never much growth at base of stab.  (F. H. (18).)

Like *armoraciae* except that the sediment in the V is stronger yellow and when 3 weeks old the whole culture is brighter yellow.  (L. McC.)
Moderate, pale yellow; sometimes slightly viscid.  (F. H. (18).)
White chemical film.  (L. McC., Rapp (86).)

Like *armoraciae*.  (L. McC.)
Surface ring nearly white.  (Rapp (86).)
In 3 to 4 weeks, yellowish rim; partial to complete pellicle; viscid, yellow precipitate.  (F.H. (18).)
Copious, primuline yellow in 9 days.  (F. H. (18).)

Liquefaction prompt.  (F. H. (18).)
Liquefaction slow.  (EFS. (34), Rapp (86), Dodge (6), Wolf (37).)
Liquid cloudy.  (Rapp (86).)
Liquefaction rapid.  (Sharp (81).)

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* Johnstone, J. R.  Unpublished notes of work done in 1906.
<table>
<thead>
<tr>
<th>Specifications</th>
<th>Bacterium campestrum armoricanae, n. var.</th>
<th>Bacterium campestrum (Pam.) EFS.</th>
<th>Bacterium phaseoli EFS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood serum (Loeffler’s No. 8)</td>
<td>At 18°C, bright-yellow growth; liquefaction begins promptly but requires 4 weeks to change all the medium to a transparent, amber-colored liquid; tyrosine crystals form.</td>
<td>No liquefaction. (Pam. (81).) Slow liquefaction. (EFS. (34), Dodge (6).) Liquid part, pale brownish. (EFS. (38).)</td>
<td>Liquefaction better than in Bact. campestrum. (EFS. (35).) Liquefaction begins in 6 to 9 days, not complete in 24 months. (F. H. (15).) In 20 days liquefaction less than in Bact. campestrum. (EFS. (34).)</td>
</tr>
<tr>
<td>Milk</td>
<td>At 28°C, rapid clearing complete in 2 to 4 days; in 2 weeks translucent throughout with small amount of submerged translucent material; narrow yellow rims; moderate yellow sediment. In 16 days the pH changed from 5.9 to 6.6; later to 7.0. After 8 weeks the cultures are like Bact. campestrum and Bact. phaseoli. Tyrosine crystals form.</td>
<td>At 28°C, slow clearing; in 2 weeks upper half of milk transparent; lower half soft, opaque curd. (L. McC.) In 16 days the pH changed from 5.9 to 6.6; later to 7.1. (L. McC.) No acid; casein settles slowly. After some weeks is partially redissolved. (EFS. (54).) No acid; casein sometimes peptonized without apparent curdling. (Hard. (17).) Tyrosine crystals form. (EFS. (34), L. McC.).</td>
<td>At 28°C, slow clearing, usually like Bact. campestrum but sometimes slower; one strain cleared in 7 days. (L. McC.) In 16 days the pH changed from 5.9 to 6.4; later to 7.0. (L. McC.) No acid; casein thrown down slowly. (EFS. (53), Rapp (56), Dodge (9).) Like Bact. campestrum. (EFS. (53).) Tyrosine crystals form. (EFS. (53), L. McC.)</td>
</tr>
<tr>
<td>Litmus milk</td>
<td>Not blued; reduction of litmus sometimes complete in 6 days, sometimes not entirely reduced in 3 weeks or more; color returns rather promptly; after 8 weeks the appearance is practically the same as Bact. campestrum and Bact. phaseoli cultures of the same age.</td>
<td>The blue color slowly deepens for 6 to 10 days; still blue in 20 days; later the litmus is more or less reduced. (EFS. (55).) Most of litmus reduced in 25 days; less rapid reduction than in var. armoraciae. (L. McC.)</td>
<td>All color disappears in 1 to 2 days. (F. H. (15).)</td>
</tr>
<tr>
<td>Methylene blue milk</td>
<td>All color reduced in 4 days; did not return in 4 months.</td>
<td>Reduction slightly slower than in armoraciae. (L. McC.) Marked reduction, one-half reduced in 9 days; in 36 days green; precipitate not stained. (EFS. (55).)</td>
<td>Reduction slower and less complete than in Bact. campestrum, but color returns more rapidly. (L. McC.)</td>
</tr>
<tr>
<td>Methylene blue in Dunham’s solution</td>
<td>Practically complete reduction of color in 10 days; color returns slowly.</td>
<td>6 days, no reddening; 9 days, distinct pink; 16 days, geranium red; 21 days, geranium red; 37 days, poppy red. (EFS. (55, 54).)</td>
<td>6 days, no reddening; 9 days, distinct pink; 16 days, geranium red; 21 days, geranium red; 37 days, poppy red. (EFS. (55, 54).)</td>
</tr>
<tr>
<td>Dunham’s solution plus rosalic acid.</td>
<td></td>
<td>15 days, greenish blue; 20 days, very pale green; 24 days, color gone; remained reduced. (EFS. (53).)</td>
<td>15 days, greenish blue; 20 days, very pale green; 24 days, color gone; remained reduced. (EFS. (53).)</td>
</tr>
<tr>
<td>Dunham’s solution plus indigo carmine.</td>
<td></td>
<td>Bacterial growth becomes red</td>
<td>Bacterial growth much as in Bact. campestrum, but often orange red. (L. McC.)</td>
</tr>
<tr>
<td>Beef agar plus Congo red...</td>
<td>Bacterial growth becomes red</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Tolerance of NaCl in beef bouillon.

Growth within 48 hours in 2, 3, and 4 per cent; growth in 14 days in 5 per cent.

Beef broth over chloroform.

Good growth in 48 hours if rather heavily inoculated; no growth if lightly inoculated.

Reaction acid and to alkali.

In beef-infusion broth: pH range, 5.3 to 9.1; pH optimum, 7.2 to 7.4. In beef-extract broth: pH range, 5.1 to 9.6; pH optimum, 6.0 to 6.6.

Potato cylinders (moist).

Pale yellow, rapidly changing to wax yellow; copious, smooth, shining growth, covering the potato and invading the liquid; in age various brownish colors; small golden-brown crystals often abundant.

Cohn's solution.

No growth.

Fermi's solution.

Very moderate, uniform clouding, milky; scanty translucent sediment.

Uschinsky's solution.

Thin, uniform clouding in 2 weeks; thin, yellow rims; moderate sediment; in 3 weeks moderate growth, rather heavy at surface as small pseudogloeospora; no fluorescence.

Temperature relations.

Optimum, 28° C. to 30° C.; maximum, 38° C.; minimum, below 2° C.; thermal death point, about 51° C.

Optimum, 30° C. or thereabouts; maximum, 38° C. to 39° C.; minimum, 5° C. or thereabouts; thermal death point, about 51° C. (EFS. (54)).

Maximun, feebly or not at all at 37° C. to 38° C.; minimum, below 7° C.; thermal death point, 51.5° C. (EFS. (55)).

Thermal death point, 48° C. (Manns (18)).

Optimum, 30° C. to 32° C. (L. McC.).

Color characters as in armoraciae. (L. McC.).

Growth limit in 3 per cent (17 days' observation). (L. McC.).

Growth limit 4 per cent. (F. H. (15)).

No growth even when heavily inoculated. (L. McC.).

Slow, long-continued growth, but with much difficulty in getting started. (EFS. (55)).

No growth in nutrient broth containing more than 3 per cent acid. (Rapp (85)).

In beef-infusion broth: Range, 8.4 to 5.7 (73 to 2.6). (Q. and F. (65)).

Like armoraciae. (L. McC.).

Like Bact. campestris. (EFS. (88), Doidge (6), F. H. (15)).

Like Bact. campestris. (EFS. (88), Doidge (6), F. H. (15)).

Feeble growth. (EFS. (55)).

Feeble growth; no pellicle; yellower and rather better growth than in Bact. campestris. (EFS. (55)).

Faint to moderate clouding. (F. H. (15)).

Growth slow or not at all. (Doidge (6)).

Thermal death point:

Approximately 46° C. (EFS. (54)).

49.5° C. (EFS. (55)).

Approximately 50° C. (EFS. (55)).

48° C. (Manns (18)).

About 50° C. (Rapp (85)).

50° C. (Wolf (37)).

45° C. to 50° C. (Doidge (6)).

Grows well between 25° C. and 30° C. (Doidge (6)).

Color characters as in armoraciae; sometimes brighter yellow than either Bact. campestris or armoraciae. (L. McC.).

Wax yellow to chrome; paler than Bact. campestris on nutrient starch jelly; on turnip and radish cultures no brown pigment. (EFS. (55)).

Chromogenesis.

Yellow, not photogenic, not fluorescent; white to pale yellow, becoming wax to apricot yellow on beef media; on potato, pale yellow becoming wax yellow and when old various shades of brown.

Color characters as in armoraciae. (L. McC.).

Wax yellow to chrome; paler than Bact. campestris on nutrient starch jelly; on turnip and radish cultures no brown pigment. (EFS. (55)).

Fuller's scale.
**Table 1.**—Morphological, cultural, and physiological comparisons of Bacterium campestris armoraciae, Bact. campestris, and Bact. phaseoli—Continued

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Bacterium campestris armoraciae, n. var.</th>
<th>Bacterium campestris (Pam.) EFS.</th>
<th>Bacterium phaseoli EFS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of indol</td>
<td>Small quantities produced in Dunham's solution, by Ehrlich's and also Salkowski's tests; Bacillus coli as check.</td>
<td>Indol formed. (EFS. (55, 54), Hard. (11), Doidge (5).)</td>
<td>No indol found in Uschinsky plus peptone. (F. H. (15).)</td>
</tr>
<tr>
<td>Production of hydrogen sulphide</td>
<td>Moderate production in beef agar plus basic lead acetate.</td>
<td>Less evidence of H₂S in basic lead acetate agar than in <em>armoraciae</em>. (L. McC.)</td>
<td>Less evidence of H₂S in basic lead acetate agar than <em>Bact. campestris</em>. (L. McC.)</td>
</tr>
<tr>
<td>Production of ammonia</td>
<td>Moderate production in beef gelatin, beef broth, and potato cultures.</td>
<td>Ammonia produced. Hard. (11. .)</td>
<td>Ammonia produced from beef broth. (F. H. (13).)</td>
</tr>
<tr>
<td>Relation to oxygen</td>
<td>Aerobic; tested in fermentation tubes with various media suitable for growth and also in shake-agar cultures.</td>
<td>Aerobic; tested in fermentation tubes with various media. (EFS. (34), Doidge (6), Hard. (11).)</td>
<td>Aerobic. (EFS. (35), Wolf (37), Doidge (6), Rapp (30).)</td>
</tr>
<tr>
<td>Diastatic action</td>
<td>Strong; starch-agar plates on fifth day had band 20 to 25 mm. wide free from starch. All starch reduced in potato cylinder cultures.</td>
<td>Same as <em>armoraciae</em>. (L. McC.) All starch reduced in potato cylinder cultures. (EFS. (35), L. McC.)</td>
<td>Good but less than in <em>Bact. campestris</em>: in 5 days the starch-free band was only 14 to 15 mm. wide (L. McC.). Strong starch-destroying action. (EFS. (35), F. H. (15), Rapp (30), Doidge (6), Sharp (31).) Starth reduced in potato cylinder cultures as by <em>Bact. campestris</em>. (EFS. (35).) On nutrient starch jelly less starch converted in 7 days than by <em>Bact. campestris</em>. (EFS. (35).)</td>
</tr>
<tr>
<td>Nitrates</td>
<td>Nitrate not reduced; no gas formed.</td>
<td>Nitrate not reduced. (EFS. (35, 54), Hard. (11), Doidge (6), L.McC.)</td>
<td>Nitrate not reduced. (EFS. (35, 54), Doidge (6), Wolf (37), Rapp (30).)</td>
</tr>
<tr>
<td>Fermentation</td>
<td>With a beef-medium (extract or infusion) base, plus the carbohydrate to be tested and bromocresol purple as the indicator, dextrose, lactose, saccharose, glycerin, maltose, mannit, and levulose, gave no acid reaction; galactose gave a slight acid reaction. With a synthetic medium base, all of the above carbohydrates gave distinct acid reactions; the strongest reactions were from dex.</td>
<td>With a beef-medium base, various workers report no acid from one or more of the following: Dextrose, lactose, saccharose, glycerin, maltose, mannit, galactose, levulose, and dextrin. (EFS. (44), Hard. (14), Doidge (6), L. McC.) With the synthetic medium base, dextrose, lactose, saccharose, glycerin, and maninit gave acid reactions equal to that from glycerin in</td>
<td>With a beef-medium base, no acid from any of the ordinary sugars. (EFS. (39).) Dextrose and saccharose produced acid slowly. (EFS. (35).) No acid from dextrose, lactose, saccharose, glycerin. (F. H. (14), Wolf (37).) No acid from maltose, galactose, manitol, arabinose, xylose, rhamnose, dextrin, salicin, and inulin. (Wolf (37).)</td>
</tr>
</tbody>
</table>
Mar. 1, 1929

**Bacterial Leaf Spot of Horse-Radish**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beef-extract agar plus sugar</strong></td>
<td></td>
</tr>
<tr>
<td>9 per cent dextrose</td>
<td>Growth not retarded. Better than in plain agar. (EFS. (35).)</td>
</tr>
<tr>
<td>17 per cent dextrose</td>
<td>Growth retarded. (EFS. (35).)</td>
</tr>
<tr>
<td>23 per cent dextrose</td>
<td>First and second experiments, growth seriously retarded. (EFS. (35).)</td>
</tr>
<tr>
<td>9 per cent levulose</td>
<td>Slight growth. (EFS. (35).)</td>
</tr>
<tr>
<td>23 per cent saccharose</td>
<td>Growth retarded, but in 24 days copious bright yellow. (EFS. (35).)</td>
</tr>
<tr>
<td><strong>Starch jelly plus glycerin</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Peptone 4 per cent, maltose 4 per cent in distilled water.</strong></td>
<td>Fluid browned. (EFS. (35).)</td>
</tr>
<tr>
<td><strong>Vitality</strong></td>
<td>Alive in agar cultures after 17½ months at 5°C to 10°C. (EFS. (35), (34).)</td>
</tr>
<tr>
<td><strong>Desiccation</strong></td>
<td>Alive after 34 days' drying; covers made from potato cultures, also from beef broth. (EFS. (35), (34).)</td>
</tr>
<tr>
<td></td>
<td>45 hours' drying at 28°C; killed all the organisms. (Hard. (11).)</td>
</tr>
<tr>
<td></td>
<td>Some survived 10 days' drying on cover glasses. (Hard. (11).)</td>
</tr>
<tr>
<td><strong>Effect of sunlight</strong></td>
<td>Killed by 10 minutes' exposure to sunlight</td>
</tr>
<tr>
<td></td>
<td>95 per cent killed in 30 minutes; 88 per cent killed in 45 minutes. (EFS. (35).)</td>
</tr>
<tr>
<td></td>
<td>Killed in 30 minutes. (Hard. (11).)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No acid produced in sugar broths. (Dolodge (6).)</td>
</tr>
</tbody>
</table>

From dextrose, saccharose, and maltose, little change in 30 days; lactose, pH 7.1 changed to 7.6 in 30 days; levulose, pH 6.2 changed to 6.8 in 30 days; galactose produced acid. (Sharp (31).)

With the synthetic medium – base, dextrose, lactose, saccharose, glycerin, maltose, levulose, and galactose produced acid. (F. H., unpublished notes.)

The various workers report no gas; no clouding in the closed arm of fermentation tubes.

**Like Bact. campesire.** (EFS. (35).)

Growth retarded but in 6 days one-half more growth than in Bact. campesire. (EFS. (35).)
First experiment, growth prevented; second experiment, growth retarded. (EFS. (35).)
No growth. (EFS. (35).)
Growth not retarded; better than in Bact. campesire. (EFS. (35).)
Growth retarded; in 24 days much less growth than in Bact. campesire and no distinct yellow color. (EFS. (35).)
Fluid not browned. (EFS. (35).)

Under similar conditions dead in one-half month (EFS. (35).)
After 27 days' drying 3 out of 9 covers made from potato cultures were alive; none out of 12 covers from beef broth were alive; seems to be less resistant to dry air than Bact. campesire. (EFS. (35).)
Alive after 70 days on glass covers. (Rapp (26).)
Alive after 18 days on glass covers. (Wolf (37).)
Alive for over 200 days when dried on glass cover slips. (Edg. (6).)
All killed in 45 minutes. (EFS. (35).)
Not all killed in 30 minutes. (EFS. (34).)
All killed in 30 minutes. (Rapp (26).)

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*NH₄H₂PO₄, 1 gm.; KCl, 0.2 gm.; agar, 15 gm.; water, 1,000 c. c.

* In January, 1927, cultures of the horse-radish bacteria were sent to George K. K. Link, with other cultures (bacteria). In a letter to E. F. Smith, May 16, 1927, Doctor Link states: "Very clearly it [the horse-radish organism] is serologically closely related to Bact. campesire. It is also related to Bact. phaseoli, but much less closely."
Rough colonies used to inoculate horse-radish leaves produced infections, but the pathogenic power of the organisms was apparently poor. Isolations from the lesions produced by rough colonies produced only smooth colonies.

In one set of plates, Bacterium campestre, isolated from spots produced by it on horse-radish leaves, produced only rough colonies, entirely similar in appearance to the rough colonies of the horse-radish bacteria. Some weeks later, in a second trial, isolation plates of Bacterium campestre from spots on horse-radish produced only smooth colonies and thus ruined the theory that an uncongenial host was responsible for the atypical colonies.

DATA ON THE HORSE-RADISH ORGANISM COMPARED WITH BACTERIUM CAMPESTRE AND BACT. PHASEOLI

Table 1 contains all the morphological, cultural, physiological, and serological data that the writer has been able to find in the publications of the various workers on Bacterium campestre and Bact. phaseoli. Every known available publication has been examined. Many of these, being mostly devoted to phases of the diseases other than the characters of the bacteria, have not been included in the bibliographical list. In this table all the characters of the horse-radish organism were determined by the writer. Some of the characters of Bacterium campestre and Bact. phaseoli were also determined by the writer in parallel tests with the horse-radish organism. In all cases the names or initials of the worker responsible for the determination of a character follow immediately after each statement.

TECHNICAL DESCRIPTION

Bacterium campestre armoraciae, n. var.

Short nonspore-forming rod; 0.7 to 2 by 0.3 to 0.5 μ, single or in pairs, on 48-hour-old beef-agar culture. Long chains form in beef broth plus 3 to 5 per cent NaCl. Motile by means of a single polar flagellum three to six times the length of the rod, stained from 48-hour-old beef-agar culture with Casares-Gil's stain; capsules; aerobic; Gram-negative; not acid-fast. Produces acid but no gas from dextrose, lactose, saccharose, glycerin, maltose, mannit, galactose, and lévulose. Does not reduce nitrates. Liquefies gelatin and blood serum slowly. Milk is readily cleared without formation of curd. Abundant production of tyrosine crystals. Litmus in milk is slowly and irregularly reduced, not blued. Scanty growth in Uschinsky's solution, slightly better in Fermi's solution, and no growth in Cohn's solution. Beef-agar colonies are wax yellow, round, smooth, shining, translucent, flat, with entire margins; structure typically uniform but sometimes delicately striated or mottled. Beef broth moderately and evenly clouded, sometimes thin yellow rims and pellicles form. Ammonia, indol, and hydrogen sulphide are produced in small quantities. Strong diastatic action on starch. Optimum temperature, 28° C. to 30° C.; maximum, 36° C.; minimum, below 2° C.; thermal death point near 51° C. Pathogenic to horse-radish, causing numerous leaf spots.

SUMMARY

This paper describes a disease of horse-radish caused by Bacterium campestre armoraciae, n. var., not heretofore described. The disease is at present known to occur in Virginia, the District of Columbia, Connecticut, Missouri, and Iowa.

The causal organism has been isolated, and its pathogenicity has been proved by inoculation experiments. Infection is chiefly, probably always, through the stomata, producing definite lesions
in the parenchyma tissue. Numerous observations and isolation and inoculation experiments have failed to demonstrate any vascular infection.

In morphological and cultural characters the organism is very similar to *Bacterium campestre* and *Bact. phaseoli*, but it differs in its host relations and reactions.

Infections, in the form of leaf spots, due to the horse-radish bacteria can be forced on cabbage, cauliflower, and bean. The lesions remain small, the infection does not spread, and the character of the infection is unlike that caused by *Bacterium campestre* or *Bact. phaseoli*. Vascular infection did not occur on these plants from inoculation with the horse-radish bacteria.

Leaf-spot infections due to *Bacterium campestre* and to *Bact. phaseoli* can be forced on the horse-radish, but even under favorable conditions infections were seldom obtained. The leaf spots when produced are few in number but very similar in appearance to those produced by the horse-radish bacteria. No vascular infection occurred.

In general, the horse-radish bacteria grow more vigorously in most culture media than either *Bacterium campestre* or *Bact. phaseoli*, and the few cultural differences may possibly be explained by this fact.

The three organisms compared are not separable or are very slightly separable by morphological or cultural characters, as determined by present methods, but they do show specific host reactions.

Cultural characters and a technical description of the causal organism are given and also a table comparing the characters of the horse-radish organism (*Bacterium campestre* var. *armoraciae*) with those of *Bact. campestre* and *Bact. phaseoli*, as noted by the writer and other workers.

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