Distribution of Bacteria Within the Tissue of Healthy Tomatoes[1]

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ABSTRACT

SAMISH, ZDENKA (National and University Institute of Agriculture, Rehovot, Israel) AND R. ETINGER-TULCZYNSKA. Distribution of bacteria within the tissue of healthy tomatoes. Appl. Microbiol. **11**:7–10. 1963.—Bacteria have been shown to be present in fresh normal tomatoes. The frequency of their presence differed widely between different fields. Bacterial populations within the tomatoes showed a distinct gradient, being largest in the connective tissue at the stem end and decreasing through the central core towards the peripheral and distal tissue. Application of *Serratia* to the sepals of young tomato fruits often resulted in their recovery from within tissue of the mature fruit. These findings lend support to the theory that the bacteria enter the fruit through the connective tissue at the stem end.

The tomato fruit represents one of the most widely studied vegetables. Abundant information has been published on the agrotechnical, chemical, and technological aspects, but only little on the bacterial content of healthy fruits (Burcik, 1950; Dawid, 1957; Schanderl, 1953; Thomas and Hobson, 1955). We found bacteria to occur quite frequently within healthy, normal, green, or mature tomatoes (Samish, Etinger-Tulczynska, and Bick, 1961). In this paper, observations are recorded as to their distribution within the fruit tissue.

MATERIALS AND METHODS

Tomatoes were collected from fields located in the vicinity of Rehovot, Israel, picked individually with stem-ends attached, and brought to the laboratory within 1 to 2 hr after harvesting. They were washed with detergents, disinfected, and flamed as described in our previous paper (Samish et al., 1961). Swabs from the surface of the treated tomatoes remained generally sterile; in a few instances, we found gram-positive bacilli. The connecting tissue between the stem and fruit, the peduncle scar, hereafter called stem-depression (Winton and Winton, 1945), was exposed by removal of the stem from the disinfected tomato. The bacterial content was determined by swabbing the exposed area with sterile moist swabs and plating on yeast dextrose agar. Pieces from the inner fruit pulp were dissected and dispersed as described previously (Samish et al., 1961).

In a second trial, the bacterial content of the tomato tissue was examined by swabs to obtain information on the location of the bacteria inside the fruit. This method was selected because it excluded practically all danger of air contamination, even though it did not allow proper quantitative determination. Fresh green tomatoes were selected, since riper fruit was too soft for this technique.

Seven locations within the fresh tomatoes were examined by cutting the disinfected flamed fruits horizontally at three levels with sterile knives (Fig. 1). In locations I and IV, approximately one-third of the circumference of the tomato was smeared with the swab. In locations II and V, the swab was plunged into the jelly of two or three locules, and in locations III and VI the entire area of the central core, including the vascular bundles, was smeared with the swabs.

When only single colonies grew on such a streak, they could have represented either small populations or they might have originated by chance from sources other than the tissue. But with this technique, there was no difficulty in distinguishing between single typical airborne colonies, which were found very rarely, and other bacteria, which usually had a higher number of colonies.

Sometimes 20 to 100 colonies of one species grew on one smear, which seemed to represent a reasonable proof of their origin in the tomato. This smear method tests, however, only a very small part of each tomato, and fruits with a low bacterial count may not have been discerned.

To study how bacteria might possibly enter into the fruits, two to three drops of *Serratia* suspension were applied upon the outer side of the sepals of young Marmand tomatoes in a field located near the laboratory. The *Serratia* suspension was obtained by adding 10 ml of sterile water to a culture growing on agar slants. Only well-shaded fruits were selected, so as to prevent rapid drying of the cultures. The tomatoes were harvested within 1 to 5 weeks after treatment, and their content of bacteria was determined after surface disinfection. In addition to the usual technique (Samish et al., 1961), pieces of the inner pulp contained in a sterile jar were covered with 30 ml of Ashby solution (Salle, 1954) and incubated for 3 days at 30 C.

Identification of the bacteria was based on *Bergey's*

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TABLE 1. *Bacteria in pulp and stem-depression of fresh tomatoes*

Date	Variety	No. of tomatoes	No. of tomatoes containing bacteria in:			No. of tomatoes with identical bacteria in both tissues
			Stem-depression	Tomato pulp	Stem-depression and pulp	
Dec. 1959	Marmand	52	22	2	9	8
Jan. 1960	Marmand	68	27	5	14	10
Jan. 1960	Moneymaker	30	7	0	4	2
Feb. 1960	Marmand	72	21	5	22	21
Total		222	77	12	49	41
Per cent of total			35	5	22	

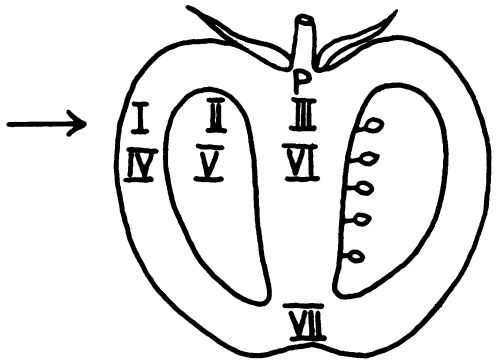

FIG. 1. *Schematic drawing of tomato, indicating locations tested.* P, *entire peduncle scar.* I, II, *and* III, *0.5 to 1 cm below stem-scar;* I *and* IV, *2 to 3 cm around periphery.* IV, V, *and* VI, *2 to 3 cm below stem-scar;* II *and* V, *inside locular jelly (at two or three spots).* VII, *0.5 to 1 cm above blossom-end;* III, VI, VII, *covering surface of central core, including vascular bundles. Arrow indicates direction of the cut.*

Manual of Determinative Bacteriology (Breed, Murray, and Smith, 1957).

RESULTS

Among 222 tomatoes examined between December 1959 and February 1960, 84 were sterile. Bacteria were found in 62% of the tomatoes, 35% in the stem-depression and about 27% in the inner tomato pulp (Table 1). In 12 fruits, bacteria were found within the pulp but not in the stem-depression. In 41 of 49 tomatoes, the bacterial species within the fruit were identical with those in the stem-depression.

The stem-depression contained (frequency given in parentheses) *Xanthomonas* (95) bacteria (yellow pigment not soluble in nutrient media, at times two or three species of the same genus); *Pseudomonas*, green pigment soluble in nutrient media (14); *Enterobacteriaceae*, mostly genus *Aerobacter* (7); *Corynebacteriaceae* (16); *Bacillus* (5); *Micrococcaceae* (2); unidentified bacteria, apparently also belonging to the *Pseudomonadaceae* family (11); yeasts (2); and fungi (5).

The concentration of *Pseudomonadaceae*, *Enterobacteriaceae*, and *Corynebacteriaceae* was often as high as 20 to 100 colonies per streak, but only single colonies of *Micrococcaceae*, *Bacilli*, yeasts, or fungi grew.

The distribution of bacteria in different parts of tomatoes, collected from six farms, was observed by examining the stem-depression as well as seven locations within the fruit (*see* Fig. 1). Among the 250 tomatoes examined with this technique, bacteria were found in the pulp of 59 fruits, i.e., about 23% (Table 2). In the stem-depression they appeared again more often, i.e., in about 58% of the fruits. The central core, just below the stem-depression

TABLE 2. *Occurrence of bacteria in different parts of the tomato*

Farm* Variety Time of harvesting	A Marmand 3-16 to 3-22	B Marmand 2-25 to 3-1	C Marmand 5-29 to 6-13	D Marmand 6-15 to 7-6	E Marmand 7-10 to 7-27	E Tamar 7-13 to 8-2	F Moneymaker 8-12 to 8-18
No. of tomatoes							
Total examined	12	12	43	70	49	44	20
Containing bacteria	7 (58%)	2 (17%)	21 (49%)	3 (4%)	20 (41%)	5 (11%)	1 (5%)
Containing bacteria in:							
Location† I	0	0	9	1	6	0	0
II	2	1	8	1	6	2	0
III	5	2	16	3	16	2	1
IV	1	1	5	0	5	0	0
V	2	1	4	0	6	0	0
VI	4	2	15	0	17	2	1
VII	1	0	8	0	2	0	0
Stem-depression of tomatoes (location P)							
Total examined	12	12	23	55	39	36	20
Containing bacteria	11	10	16	9	33	30	6
Containing more than ten colonies per streak	9 (75%)	2 (17%)	12 (52%)	4 (7%)	26 (67%)	18 (50%)	2 (10%)

* Farms B, D, and F were irrigated in furrows; farms A, C, and E had overhead irrigation.
† See Fig. 1.

(locations III and VI, Fig. 1), showed the highest frequency of bacteria, i.e., 86 times. Bacteria were found in the locular jelly (locations II and V) 33 times, but in the tissue adjacent to the peel (locations I and IV) 28 times. In location VII, i.e., about 0.5 cm inside the stylar-end, bacteria occurred in only 11 of 250 tomatoes.

Conspicuous differences were recorded between fruits derived from different farms. Tomatoes from three of the farms (A, C, and E), which used overhead irrigation, contained bacteria far more frequently and at higher concentration than those from farms B, D, and F, which irrigate in furrows.

Tomatoes of the variety Tamar, grown on Farm E under identical conditions as Marmand, did not differ greatly in bacterial population of the stem-depression, but penetration of the bacteria into the fruit tissue was only about one-fourth that of the Marmand variety. These observations confirm our previous findings (Samish et al., 1961).

TABLE 3. *Concentration and identification of bacteria in different locations within tomatoes*

Location*	No. of tomatoes containing bacteria in each location	Concn of bacteria†	No. of tomatoes containing:						
			Pseudomonadaceae	*Enterobacteriaceae*	*Corynebacteriaceae*	*Micrococcaceae*	*Bacillus*	Molds	Yeasts
I	16	1–4	4	0	3	0	0	0	0
		5–20	5	2	1	0	0	0	0
		>20	3	0	2	0	0	0	0
II	20	1–4	4	1	1	0	0	0	0
		5–20	2	2	1	0	0	0	0
		>20	3	1	5	0	0	0	0
III	45	1–4	13	4	0	0	0	0	0
		5–20	15	3	1	0	0	0	0
		>20	8	2	3	0	0	0	0
IV	12	1–4	2	0	1	0	0	0	0
		5–20	2	1	0	0	0	0	0
		>20	3	1	4	0	0	0	0
V	13	1–4	4	1	0	0	0	0	0
		5–20	3	1	1	0	0	0	0
		>20	2	2	3	0	0	0	0
VI	41	1–4	15	3	0	0	0	0	0
		5–20	8	4	1	0	0	0	0
		>20	11	2	4	0	0	0	0
VII	11	1–4	5	1	1	0	0	0	0
		5–20	2	0	1	0	0	0	0
		>20	1	1	6	0	0	0	0
Stem-depression	115	1–4	25	8	3	8	11	4	1
		5–20	23	10	6	0	0	0	0
		>20	50	8	12	0	1	0	0

* See Fig. 1.
† Number of colonies per smear.

The bacteria recorded on the smears from the seven locations and from the stem-depression (Table 2) were identified, and the frequency of their occurrence was estimated (Table 3). The tomato tissue contained only representatives of three families, *Pseudomonadaceae*, *Enterobacteriaceae*, and *Corynebacteriaceae*.

The *Pseudomonadaceae* belonged mostly to the genus *Xanthomonas*. The four most abundant species were described in detail in a previous paper (Samish et al., 1961).

The *Enterobacteriaceae* belonged to the tribe *Escherichieae*, mostly to the genus *Aerobacter* (methyl red-negative, Voges-Proskauer-positive, citrate-positive). About 80% produced acid and gas at 30 C in 3 days on lactose broth, and about 20% were anaerogenic, producing only acid but no gas. Some produced pale- to gold-yellow water-insoluble pigmentation on agar, which became marked after 2 to 4 days of incubation. *Escherichia freundii* (Voges-Proskauer-negative, methyl red-positive, citrate-positive) was found only rarely. *E. coli* was never present. The *Corynebacteriaceae* (genus *Corynebacterium*) showed characteristic angular arrangement of the bacteria, were gram-positive, catalase-positive, and did not decompose cellulose. The colonies were very small, round, and varying in pigmentation (yellow-orange, lemon or mustard colored, pink or greenish); the bacteria were mostly motile, and differences in average size, growth on litmus milk, nitrate reduction, and utilization of citrate proved the presence of different species.

In the central core of the fruit (locations III and VI), as well as in the stem-depression, the dominating bacteria belonged to the family *Pseudomonadaceae*, and *Corynebacteriaceae* dominated within the tissue near the stylar-end. In an additional test of 50 tomatoes of the variety Moneymaker, however, the genus *Aerobacter* was found in 20% of the fruits, and *Pseudomonadaceae* in only 4%. On one farm (farm E), we found a local strain of *Corynebacteriaceae* producing pink colonies, which were not found in tomatoes from other farms.

The 59 tomatoes which contained bacteria may be divided, according to the bacterial distribution in their tissues, into four groups.

Group A (27 tomatoes). Bacteria were concentrated only in the central core; they were generally identical

TABLE 4. *Recovery of Serratia in tomatoes after the application of suspensions upon the fruit sepals*

Days between application and harvest	No. of tomatoes examined	No. of tomatoes containing *Serratia* in:		
		Stem-depression	Fruit pulp	Stem-depression and fruit pulp
6–10	13	2	1	4
11–20	9	2	1	5
21–40	18	2	0	5
Total	40	6	2	14

with the microflora of the stem-depression itself. In a few rare instances, the bacteria were found in the fruit core only, but not in the stem-depression, or different bacteria were recorded in each.

Group B (*17 tomatoes*). Bacteria were distributed inside the fruit, in the central core, as well as in the locular jelly, and at times also in the peripheral tissue, but not in the stylar-end.

Group C (*11 tomatoes*). Bacteria, mainly *Corynebacteriaceae*, were present in these fruits in the stylar-end and often also in other tissue, but not in the stem-depression; these bacteria may possibly have spread into the tissue from the stylar-end.

Group D (*4 tomatoes*). Bacteria were found only at the periphery of the fruit (locations I and IV). Possibly, fruits with damaged peels had been accidentally included in the tests, despite all precautions, and some injuries not visible to the eye may have served as means of entrance to the microflora.

A number of tests were conducted to determine how bacteria penetrate into the tomato. *Serratia* were selected, since we never found them inside the tomatoes or stem-depression, and since they can be quite readily identified. If cultures of the bacteria were smeared upon the peel of young tomatoes growing in the field, the bacteria could not be recovered later inside the fruit. If they were applied with a platinum needle below the skin, the fruit which had been punctured developed normally and the bacteria could be recovered generally only in the canal of puncture in the pulp. But when suspensions of *Serratia* were applied to the sepals of young Marmand fruits, these bacteria were recovered in 22 of 40 fruits (Table 4).

Their recovery in more than one-half of the treated fruits indicates that the microorganisms may enter the fruit in the vicinity of the sepals.

DISCUSSION

The stem-depression, which is exposed after the tomato stem is pulled off, is very often populated by bacteria, more so than the underlying fruit pulp. This fact suggests that bacteria may penetrate into the inner pulp from this area. Bacteria belonging to the same families as those found in the stem-depression are quite commonly found on the leaves of the tomato plant, on the sepals, and, at times, also upon the surface of the tomato itself.

Thus, it could well be envisaged that some bacteria find a suitable medium for survival and multiplication in the stem-depression, and penetrate from there into the growing fruit. Such a theory also finds support from two of our findings: (i) *Serratia* suspensions, which had been applied upon the sepals of growing tomatoes, were later recovered in the stem-depression, and often also in the fruit pulp; and (ii) the central core of the tomato has a higher bacterial concentration than the peripheral tissue (except the stem-depression).

Representatives of the two bacterial families most often found within the tomatoes belong to the normal epiphytal flora of these plants. They presumably progress into the fruit tissue more readily than other members of this flora because of their comparatively smaller size and motility.

A yellow water-insoluble pigment was produced on agar by several strains of the genus *Aerobacter*. Such yellow-pigmented coli-aerogenes bacteria were also isolated by Thomas and Hobson (1955) from cereal crops. *Coccaceae*, which had been found in tomato tissue in Germany by Burcik (1950), were, however, rare in tomatoes grown in Israel.

The considerable fluctuations in bacterial content of the tomatoes obtained from different fields could be due to a number of factors, such as varietal characteristics, climatic influences, or agrotechnical practices.

When Marmand tomatoes were grown in the same experimental field, in parallel plots with Tamar tomatoes, bacteria occurred in the stem-depression of both varieties with similar frequency, but the bacterial content of their fruit pulps differed conspicuously, due possibly to anatomical differences between the two varieties.

Marmand tomatoes harvested between January and March, and again between June and July, were of similar bacterial content, but conspicuous differences were obtained between samples from nearby farms in the same season. Possibly the differences in methods of irrigation may be responsible, with overhead irrigation producing a microclimate more favorable to bacterial development.

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