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and *E. coli* indices, respectively, of water samples, and this method should be equally applicable to food products.

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Pustule Formation by *Lactobacilli* on Fermented Vegetables

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Genuine dill pickles are the product resulting from the fermentation, predominantly lactic, of cucumbers immersed in a brine containing 3 to 4 per cent salt. The brine also contains dill weed and may contain spices and garlic or extracts of their essential oils. Genuine dill tomatoes are manufactured from green tomatoes by a fermentation similar to that used for pickles (Fabian and Wadsworth, 1944).

Lactobacillus plantarum is commonly associated with the fermentation of pickles (Pederson, 1936), although other lactobacilli and some yeasts undoubtedly play a role. A survey of the literature failed to uncover any reports concerning the microbial flora of fermenting dill tomatoes. Pederson (1936), however, mentions that *L. plantarum* has been found in fermenting and spoiled tomato products.

The formation of white pustules on fermented olives has been investigated by Vaughn *et al.* (1953). These workers found that the pustules were actually massive, subepidermal growths of *L. plantarum*. They also noted, but did not investigate, the presence of such pustules on fermented Italian peppers and pickled green tomatoes.

We have noted the presence of white pustules on commercially packed dill tomatoes and pickles and on sweet pickles prepared from salt stock. This report deals with microbiological and histological studies of pustules on dill tomatoes and pickles.

MATERIALS AND METHODS

Isolation and Study of Microorganisms

Isolations were made from home-canned dill pickles and dill tomatoes 2 to 3 months after active fermentation had ceased. The tomatoes or pickles were washed

in tap water, immersed for 10 sec in a solution containing 50 ppm of chlorine, and rinsed in tap water. Pustules were lanced with a sterile scalpel, and a small amount of the pustular material was shaken thoroughly in sterile water. The aqueous suspension was streaked on tomato juice agar (Difco tomato juice agar, with the agar content increased to 2 per cent), and the plates were incubated at 30 C either aerobically or in an atmosphere of 10 per cent carbon dioxide and 90 per cent hydrogen. Controls were carried out on the efficiency of the surface sterilization and sterility of the tap water.

Well-isolated colonies were picked from the streaked plates and their purity ensured by successive streaking on tomato juice agar. Forty-four cultures were studied for gram reaction, catalase production, and ability to ferment sugars and polyalcohols. The following compounds were added aseptically to 0.5 per cent sterile yeast extract broth containing brom cresol purple indicator in order to yield a final concentration of 0.5 per cent: glycerol, inulin, maltose, mannitol, arabinose, xylose, fructose, galactose, glucose, mannose, lactose, sorbitol, sucrose, rhamnose, and raffinose.

Yeast extract or 2 per cent malt sprout extract containing 3 per cent glucose was employed as the medium for the production of acid by representative cultures. Qualitative and quantitative studies were made on the fermented medium 4 to 7 days after inoculation.

Tests for volatile acids were made by steam distillation of the acidified fermented medium and titration of the distillate with standard base (Neish, 1952). Lactic acid was recovered as zinc lactate by the method of Brin, Olson, and Stare (1952), and the rotation determined polarimetrically.

Quantitative determinations of acid were made by

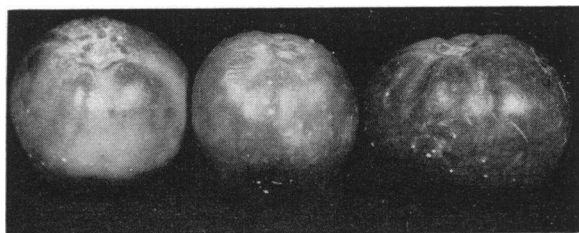


FIG. 1. Pustules on tomatoes fermented for two months in brine.



FIG. 2. Pustules on cucumbers fermented for two months in brine.

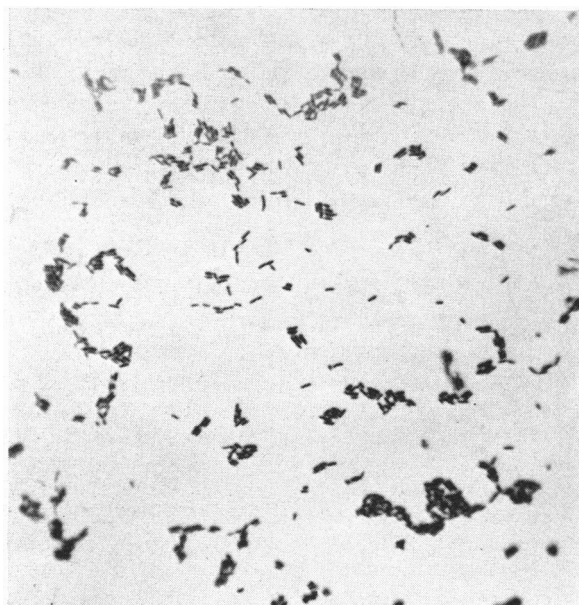


FIG. 3. Bacteria taken directly from pustular material in tomato ($\times 1000$).

titrating with standardized base or by the colorimetric method of Barker and Summerson (Neish, 1952).

Histology

Histological studies of vertical sections through the pustules were made. Blocks of material containing pustules were embedded, sectioned, and stained with hematoxylin according to methods described by Sass (1951).

EXPERIMENTAL RESULTS

Pustules and Bacteria

During the two successive years this work was carried on, pustules were observed on the majority of tomatoes

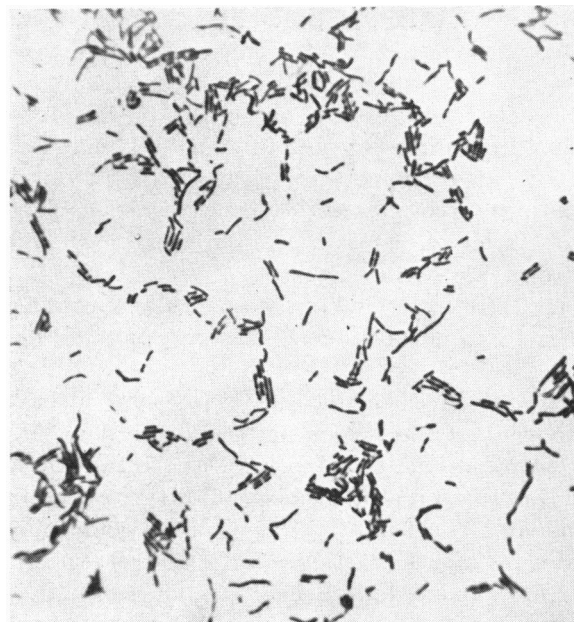


FIG. 4. Bacteria taken directly from pustular material in cucumber ($\times 1000$).

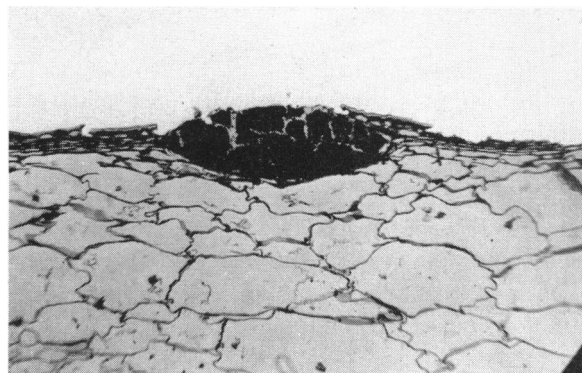


FIG. 5. Vertical section through pustule on tomato ($\times 50$)

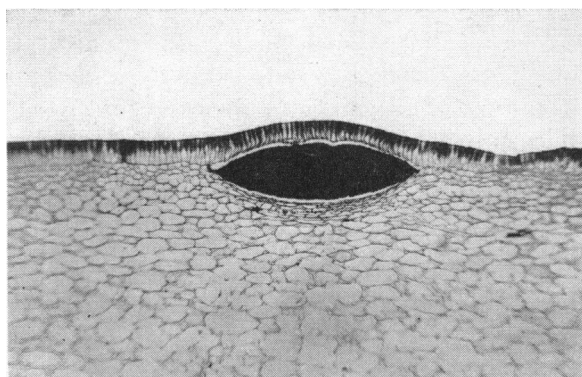


FIG. 6. Vertical section through pustule on cucumber ($\times 50$)

and on a number of the pickle samples examined. Small, white, convex blemishes appeared approximately 3 weeks after the initiation of fermentation. Within 6 weeks, the pustules were very pronounced and had attained a maximum diameter of from 0.5 to 3.0 mm (figures 1 and 2). On incision of the epidermis covering the

pustules, a white butyrous material, composed of gram-positive, rod-shaped bacteria, was obtained (figures 3 and 4).

Forty-four cultures were obtained from 17 tomatoes selected from three batches of fermented tomatoes. All of the isolates were catalase-negative, and all failed to produce gas from any of the carbohydrates employed. Volatile acid determinations on fermented media failed to indicate even trace amounts of acetic acid. On the basis of these results, all of the isolates were considered to belong to the homofermentative group of *Lactobacillus*.

Thirty-seven of the isolates were found to have a carbohydrate fermentation pattern identical to that of *L. plantarum*. Lactic acid isolated as the zinc salt was of the inactive form. All members of this group produced abundant lactic acid (1.5 per cent). The remaining isolates resembled *L. leichmannii* in all respects except that they produced only small amounts of lactic acid (0.4 per cent) in malt sprout glucose broth. No isolates were obtained from cucumbers, although material from several pustules was streaked on tomato juice agar and incubated both aerobically and anaerobically.

Histology

Vertical sections through pustules on tomatoes and cucumbers are shown in figures 5 and 6, respectively. The lense-shaped masses of bacteria have caused a compression of parenchymal cells and a bulging of the epidermis. The epidermis covering pustules in tomatoes was partially sloughed off in all sections of three pustules studied, but the relationship of the epidermis to the pustule can be seen in figure 5.

Discussion

This report extends the observations of Vaughn *et al.* (1953), who investigated only white spots on olives, to fermented tomatoes and cucumbers. In contrast to the results of these workers who found only *L. plantarum*, we have isolated two species responsible for producing pustules in tomatoes, *L. plantarum* and *L. leichmannii*. Only one species was present in a single pustule.

The presence of bacteria in pustules on cucumbers was readily demonstrated microscopically, but the failure to recover viable organisms by methods which were suitable for the studies on tomatoes is not readily ex-

plainable. Vaughn *et al.* failed to recover viable organisms from pustules on olives which had been fermented for 9 months but the cucumbers which were investigated in our laboratory had been fermented only 2 to 3 months.

The presence of these blemishes on fermented tomatoes and cucumbers is of biological interest, but probably of little economic importance. The dill pickles are not rendered unsightly, and sweet pickles made from salt stock containing pustules are acceptable.

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SUMMARY

Subepidermal, shiny white pustules on fermented tomatoes and cucumbers have been studied histologically and bacteriologically. The pustules are massive growths of gram positive, rod-shaped bacteria which compress the surrounding parenchyma and cause a bulging of the epidermis. *Lactobacillus plantarum* and *Lactobacillus leichmannii* were isolated from the pustules on tomatoes. Bacteria were observed in the pustular material from cucumbers, but no isolates were obtained by the methods used.

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