perimental work on certain phases will be continued.

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BACTERIOLOGICAL SURVEY OF SOME CITRUS CANNERIES IN FLORIDA WITH SPECIAL ATTENTION TO *ESCHERICHIA COLI* [1]

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Escherichia coli is an organism commonly associated with a certain type of bacterial contamination. The presence of *E. coli* should be given serious attention even though it is generally considered harmless because it is commonly found in the intestinal tract of warm-blooded animals. When this organism is present it is considered evidence of contamination, usually fecal, and indicates a health hazard. There is the danger that other bacteria capable of producing typhoid fever, dysentery, or other intestinal diseases may be present. The presence of *E. coli* in a food product may be determined by a series of simple tests. The American Public Health Association has recommended a standard routine check to determine the presence of *E. coli* in milk and water supplies and has been of great value in safeguarding the consumer's health. They have been a valuable aid in developing suitable sanitary measures so essential to healthful living. The results of routine analyses supported by differential tests as applied to citrus cannery equipment and unpasteurized citrus fruit products are presented in this discussion.

Samplings were made at ten citrus canneries in the Winter Haven area over a period of three years. Each plant was inspected at least once and several of them three times a season. The investigation included samples of the unwashed fruit, washed fruit, and of material from washers, conveyors, sizers, juice extractors, juice troughs, and juice blending tanks.

A brief description of the steps taken while the search for *E. coli* in unpasteurized products will be given. A known dilution is made with sterile water from the thoroughly agitated liquid portion of a sample. Petri dishes of eosin-methylene blue (E.M. B.) agar are inoculated with some of the diluted sample; observations are made at the end of 18 and 24 hours of incubation at 37° C. Eosin-methylene blue agar is a selective medium. It is a mixture of lactose (milk sugar), other nutrients, and dyes. When *E. coli* grows on the surface of this medium, the dyes are incorporated with the cell growth, and a colony is formed that is distinctive in appearance for *E. coli*. The diagnosis of colonies grown on this medium requires a skill that may be devel-

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oped through careful observations and comparisons with *E. coli* grown on the same medium.

Well isolated colonies are chosen for making bacterial suspensions in sterile water blanks from which lactose (milk sugar) broth and media for differential tests are inoculated. The differential test media and the purposes they serve are as follows: Tryptone solution, the first to be considered, is digested by *E. coli*, and indol is formed; the byproduct of growth is recognized as a color in solution when certain analytical reagents are added. Next in order is glucose solution which gives two differential characteristics. *E. coli* forms acid in glucose which is strong enough to cause methyl red indicator to turn red when added to the medium; the other characteristic sought for in this fermentation is the formation of acetoin. The Voges-Proskauer test is employed, and the reagents used cause a color formation in the medium when acetoin is present. The test is negative if *E. coli* has been grown in glucose. A medium containing citric acid as the only carbon nutrient present is the last in the series, and this medium will not support the growth of *E. coli*. These differential tests are known as the "Imvic" tests. Other differential media may be added to the list but these are considered the minimum number of tests to decide the absence of *E. coli* in a product.

A brief description of *E. coli* is gleaned from cultures on these media follows: The bacterium was a short rod. Spores were not formed. The test with Gram's stain was negative. Acid and gas were formed in lactose (positive reaction). Indol was produced. Methyl red reaction was positive in glucose and Voges-Proskauer test was negative. There was no growth on citric acid mdium (negative reaction).

When these tests were applied to organisms in citrus fruit products, *E. coli* was not found among the lactose fermenting, gram-negative, nonspore-forming rods. A summary of results for "Imvic" tests made during the last three seasons showed organisms representing six groups (Table 1).

TABLE 1.

"IMVIC" TEST GROUPS OF EOSIN-METHYL BLUE AGAR SELECTED COLONIES

Group	Indol	Methyl Red	Voges-Proskauer	Citric Acid
1	−	−	+	+
2	−	+	−	+
3	+	+	+	+
4	−	−	−	+
5	+	+	−	+
6	−	+	+	−

Many of the colonies selected from eosin-methylene blue (E. M. B.) agar plates resembled *E. coli* so closely that one with little experience would be inclined to call them coli positive. The experienced worker might be inclined to call them negative, yet would feel that it was very unwise not to proceed with differential tests. It was observed that these organisms formed colonies on E. M. B. agar much more slowly than *E. coli;* incubations usually required 48 and sometimes 72 hours.

These E. M. B. selected organisms and *E. coli* are very easily destroyed by heat; therefore, a product that has been pasteurized would not show visible forms. *E. coli* inoculated into orange juice (acidity 0.7 per cent) at room temperature was not viable after four days. As the concentration of solids increased, the death rate of *E. coli* increased. At 31 per cent dry substance, as determined by the refractometer,

viable *E. coli* was not found after 48 hours, The behavior of the unidentified E. M. B. selected bacteria almost paralleled that of *E. coli.* Grapefruit sections inoculated with *E. coli* and unidentified E. M. B. selected organisms were frozen and stored at sub-zero temperatures. *E. coli* was not found after eight days, but some of the other kinds were found after two months. The effect on viability of high-solid concentrations and citrus juice in sub-zero storage has not been investigated.

It would be interesting to know about the origin and distribution of these organisms that resemble *E. coli* so closely in appearance when grown in lactose broth and on eosin-methylene blue agar. Such knowledge might be a means of preventing economic loss to the producer when a hasty inspection would otherwise lead to the condemnation of his product and his plant sanitary program. Some thought has been given to this phase of the work, but a complete story cannot be given at this time. Many of those bacteria are known to live in soil normally, and one would expect that they enter the plant with the fruit. The surface of washed fruit has been checked for this type of contamination, but the tests were negative. The conveyor system was seldom found to be contaminated with these organisms, but the extractors and the juice collected at the extractors carried these bacteria almost invariably. Fruit flies (*Drosophila*) pass these organisms alive from their bodies, and it is possible that they contribute considerable contamination to the extractors when they are feeding on micro-plant life (yeasts and bacteria) and juice. Where the fruit flies collect the contamination distributed by them has not been determined. It is also possible that fruit which seems to be normal in outward appearance has sub-surface defects due to injuries and that the bacteria in question may come from such fruit which has begun to spoil.

This paper is a brief report on eosin-methylene blue (E. M. B.) agar selected bacteria collected from ten citrus fruit processing plants in the Winter Haven area. The investigation extended over three seasons. Each plant was investigated at least once, and some of them three times during the season. *Escherichia coli* was not found in the unpasteurized fruit product examined. The significance of the E. M. B. agar selected bacteria resembling *E. coli* has not been established. Additional work should be done to determine the source and significance of these bacteria in citrus fruit processing plant.