APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1985, p. 1527–1529
0099-2240/85/061527-03$02.00/0

Vol. 49, No. 6

Klebsiella pneumoniae in Orange Juice Concentrate

FRANCISCO A. FUENTES,[1] TERRY C. HAZEN,[1]* ARLEEN J. LÓPEZ-TORRES,[1] AND PIO RECHANI[2]

Microbial Ecology Laboratory, Department of Biology,[1] and Department of Chemistry,[2] University of Puerto Rico, Río Piedras, Puerto Rico 00931

Received 3 December 1984/Accepted 19 March 1985

Fecal coliform-positive, capsule-forming *Klebsiella pneumoniae* cells were observed in high densities (10^4 to 10^8 CFU/100 ml) in two commercial batches of frozen orange juice concentrate at a cannery in Puerto Rico. Contamination of both lots was gross and included off colors and odors. Isolates of *K. pneumoniae* from these concentrates revealed growth at 4, 25, and 34°C with generation times from 0.39 to 1.84 h.

Frozen storage is commonly used to prevent microbial spoilage of foods. Freezing inhibits the growth of most food-borne microorganisms; however, spoilage often occurs in frozen citrus concentrates due to prolonged survival of acid- and sugar-tolerant psychrotrophic microorganisms (9). The microbial deterioration of orange concentrate may represent a direct health hazard to the consumer when the spoilage flora includes species of pathogenic psychrotrophs. There is a paucity of information available on the contamination of frozen citrus concentrates by potentially pathogenic bacteria and in particular by fecal coliform bacteria (5, 16). Recently our laboratory observed gross coliform contamination of crude batches of orange concentrate from a juice cannery in Bayamón, Puerto Rico. We were asked to examine the microbiological quality of this concentrate by the local cannery, since its physical appearance was suspicious to quality control personnel. Samples from 43 lots were collected and transferred to sterile Whirl-Pak bags (Nasco International, Fort Wilkinson, Wis.) by aseptic techniques and were kept on ice until processed (2 to 3 h after collection).

The lots assayed were part of two different batches of orange concentrate. Most of these lots (39 of 43) were furnished by company A in Florida. The orange juice in this batch was prepared with oranges from Texas, Brazil, and Florida. The remaining lots were part of a batch purchased from another company (Company S) in Florida and prepared with oranges from Brazil. Both batches of orange juice were concentrated by flash evaporation to the required Brix level (58° Brix [pH 3.2]). At this stage whole juice was added to the concentrate to minimize the generation of off flavors. The concentrate was dispensed into plastic bags and then tightly sealed in (189-liter) metallic barrels. After concentration and packaging, all lots were maintained under frozen storage and shipped to Puerto Rico.

Analyses for total and fecal coliform bacteria were performed by using m-Endo and m-Fc media (Difco Laboratories, Detroit, Mich.), respectively. All bacteriological procedures were performed according to standard methods (1). Bacteria were identified and confirmed by using the API 20E (Analytab Products, Plainview, N.Y.) oxidase test (filter disk method), the motility test (hanging-drop preparation), and capsule staining. Cultures were maintained in tryptic soy agar slants (Difco). A pure cell suspension of *K. pneumoniae* (strain isolated from frozen orange concentrate; API profile, 5215773) was prepared from pure cultures grown on tryptic soy broth (Difco) at 34°C. Cells were harvested during the exponential-growth phase by centrifugation and washed with a filter-sterilized 0.85% NaCl solution. The final cell concentration was adjusted to ca. 10^4 cells per ml by using a Coulter Counter model ZF (Coulter Counter, Hialeah, Fla.). For the survival study, 1-ml samples of the cell suspension were added to 250 ml Erlenmeyer flasks containing 100 ml of 10% orange concentrate (pH 3.24) (Orange Plus; General Foods Corp., White Plains, N.Y.) obtained in a local food store. Three sets of triplicates were incubated at 4, 25, and 34°C without shaking. At selected time intervals 1-ml samples were withdrawn from each flask. *Klebsiella pneumoniae* counts were determined by using McConkey-inositol-carbenicillin agar (3).

This work presents evidence on the isolation of a *K. pneumoniae* strain from undiluted frozen orange concentrate, thus confirming the ability of this bacterial species to cope with extreme environments (Table 1). The density of indicator bacteria exceeded by far the limits tolerable for "indicator" fecal contamination of foods (10^5 to 10^7 total coliforms and 10^4 to 10^6 fecal coliforms per 100 ml; Table 2). Of 20 randomly selected positive fecal coliform isolates, 18 (90%) were identified as *K. pneumoniae* (API profile, 5215773) and 2 (10%) as *Enterobacter cloacae* (API profile, 3105773) (Table 2).

K. pneumoniae is a well-known opportunistic pathogen among members of the *Enterobacteriaceae* family, causing pneumonia, urinary tract infections, and gastroenteritis (12). At the same time, *K. pneumoniae* has also been identified as a major component of the microbial flora in several types of stressed nonclinical environments (11, 17, 18, 19; A. J. López, M.S. thesis, University of Puerto Rico, Río Piedras, Puerto Rico, 1982). High densities of *K. pneumoniae* have

TABLE 1. Detection of fecal and total coliform bacteria in frozen orange concentrate samples

Batch source	No. of lots assayed	No. with total coliforms[a]	No. with fecal coliforms[b]
Company A	39	29	22
Company S	4	4	4

[a] Number of lots showing positive reactions; cells counts, $\geq 10^5$ CFU/100 ml.
[b] Number of lots showing positive reactions; cell counts, $\geq 10^4$ CFU/100 ml.

* Corresponding author.

TABLE 2. Microbiological quality of frozen orange concentrate

Lot no.	Total coliforms[a] (CFU/100 ml)	Fecal coliforms[a] (CFU/100 ml)	Isolate[b]
1400	1×10^6	1.5×10^5	*K. pneumoniae*
5196	6×10^5	1.5×10^5	*K. pneumoniae*
5207	1.5×10^6	1×10^5	*K. pneumoniae*
5208	4×10^6	1.3×10^5	*K. pneumoniae*
5210	1×10^7	3×10^6	*K. pneumoniae*
5211	4×10^5	5.4×10^5	*K. pneumpniae*
5216	6×10^6	3.6×10^5	*E. cloacae*
5258	8×10^5	6×10^4	*K. pneumoniae*
5261	2.1×10^6	1×10^6	*K. pneumoniae*
5263	1×10^7	5×10^6	*E. cloacae*
5266[c]	5×10^5	5.1×10^5	*K. pneumoniae*
5268	8×10^6	2.5×10^6	*K. pneumoniae*
5269	4×10^6	1.2×10^6	*K. pneumoniae*
5274	1×10^7	8×10^6	*K. pneumoniae*
5275	9×10^5	1×10^6	*K. pneumoniae*
5277[c]	6×10^5	2×10^5	*K. pneumoniae*
5281	1×10^7	2×10^5	*K. pneumoniae*
5291	3×10^6	1×10^6	*K. pneumoniae*
6581[d]	1.6×10^6	1×10^5	*K. pneumoniae*
6591[d]	3×10^5	7×10^5	*K. pneumoniae*

[a] Values represent the average densities of duplicate assays.
[b] Most abundant bacterial species among randomly selected positive fecal coliform isolates.
[c] Identification of isolates from this lot yielded only *K. pneumoniae*.
[d] Lot furnished by Company S. All the other lots were obtained from Company A.

been reported from aquatic environments receiving industrial wastewaters which have a low pH, high conductivity, and a high content of solutes and particulate matter (6). This bacterium has also been recovered from foods with a high content of sugars and acids (8, 14). The intimate association of *K. pneumoniae* with plants and plant by-products is also well documented (7, 13). Thus, *K. pneumoniae* is considered a ubiquitous bacterium with an unusual ability among coliform bacteria to survive under a very heterogenous set of environmental conditions, including high-acid and high-osmotic-pressure conditions.

The environment in the frozen orange concentrate is, to say the least, inhospitable: low temperatures, low water activity, and low pH (pH 3). Our isolate of *K. pneumoniae* not only survived prolonged storage in orange concentrate under freezing temperatures, but showed a typical bacterial growth curve when cultured in diluted orange concentrate over a wide range of temperatures (Fig. 1). This isolate had a generation time of 1.84 h even at 4°C (Table 3). The abundant production of extracellular products, as indicated by the slimy appearance of colonies grown on tryptic soy agar and the presence of exuberant capsules, may account for the acid and sugar tolerance of our *K. pneumoniae* strain (10, 20). It is also interesting to note that although our isolate showed a positive reaction in the fecal coliform test, it was able to survive and grow over a wide range of temperatures in the orange concentrate. Fecal coliform-positive strains of *K. pneumoniae* able to grow at low temperatures (10°C), although rare, have been reported by other (2, 4).

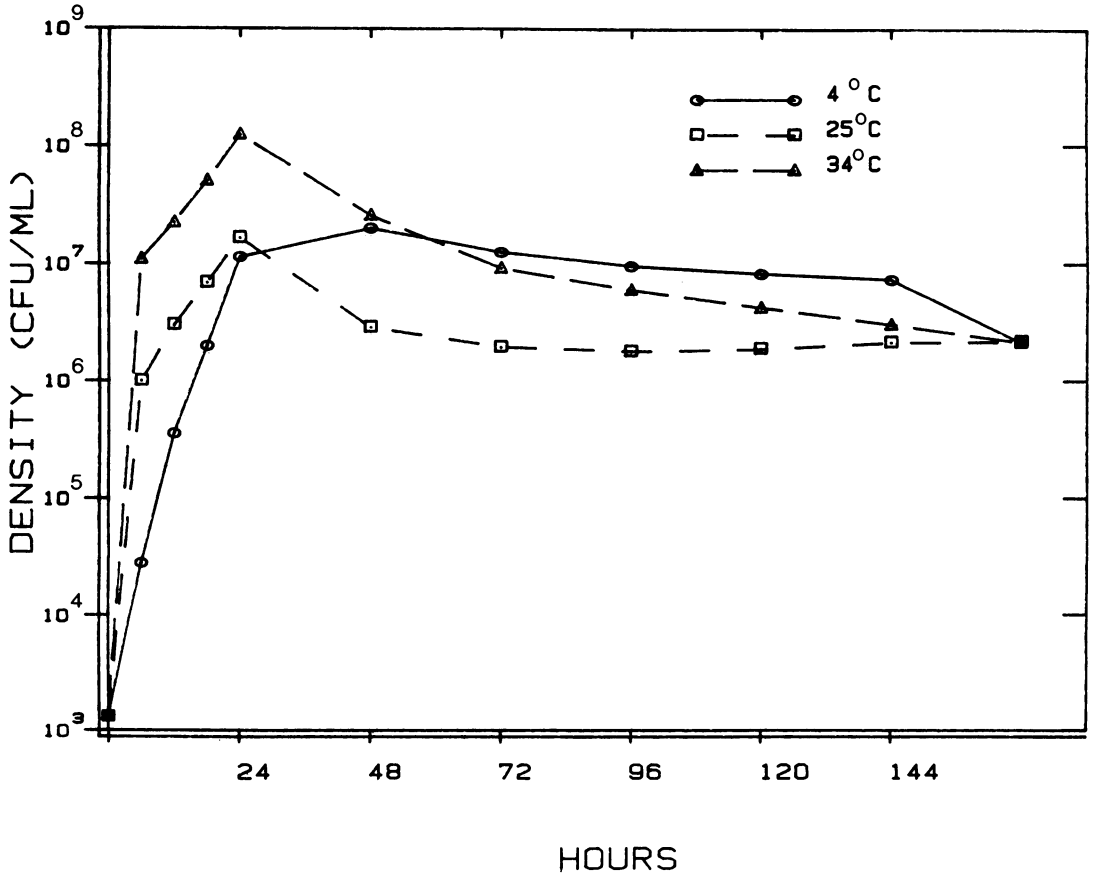

FIG. 1. Effect of temperature on growth of *K. pneumoniae* in orange concentrate.

TABLE 3. Effect of temperature on generation time and maximum cell density *K. pneumoniae* growing in orange concentrate[a]

Temp (°C)	Generation time (h)	Maximum cell density (CFU/ml)
4	1.84	2.00×10^7
25	0.48	1.65×10^7
34	0.39	1.24×10^8

[a] Initial density, 1.3×10^3 CFU/ml; fermentation odor apparent after 48 h of incubation at 25 and 34°C.

Whether the presence of *K. pneumoniae* in the commercial batchs of frozen orange juice indicates pollution of fecal origin or contamination with natural bacterial populations is difficult to determine. The fact that not even a single isolate of *Escherichia coli* was obtained from among the fecal coliform and total coliform targets suggests nonfecal contamination.

Regardless of the fecal or nonfecal origin of the bacteria isolated from the orange concentrate, several potential sources of contamination were possible. The first possibility is that contamination occurred during the extraction and collection of the raw material. Microbes on the surface of oranges and in collection vessels could contaminate the orange juice at this stage. After the concentration of the orange juice by flash evaporation, most of the contaminating flora is heat killed. Thus, the high counts of fecal coliform bacteria may represent the growth of survivors: *K. pneumoniae* and *E. cloacae*. Although *K. pneumoniae* has been recovered from tomato juice (pH 4.3) heated at 60°C for 75 min (15), it seems unlikely that this occurred in our case since flash evaporation requires even higher temperatures. The second and more likely possibility is that contamination came from the whole juice added to the orange concentrate to prevent the development of off flavors during prolonged cold storage. The third possibility is that contamination was caused by bacteria already present in the machinery used to dispense the orange concentrate. Since the isolate of *K. pneumoniae* we tested was able to survive and grow in orange concentrate at low temperatures for extended periods, both of the preceding alternatives seem plausible. The final possibility is that contamination of the concentrate occurred in the local processing plant, as the result of nonaseptic handling procedures; this was unlikely since 90% of the barrels had not been opened since leaving Florida.

Further studies will be aimed at determining the actual origin of this contaminant, since it may represent a serious threat to human health, as well as to the economy of frozen food industries.

This work was supported by Public Health Service grant RR-8102 from the National Institutes of Health and in part by the office of Fondo Institucional para Investigación, University of Puerto Rico, Río Piedras, by Sea Grant (National Oceanic and Atmospheric Administration, U.S. Department of Commerce) UPR SG 04F15844030 project EN/P-45, and by the Water Resources Research Institute of the University of Puerto Rico at Mayaguez (U.S. Department of the Interior).

LITERATURE CITED

1. **American Public Health Association.** 1981. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Inc., New York.
2. **Bagley, S. T., and R. J. Seidler.** 1977. Significance of fecal coliform-positive *Klebsiella*. Appl. Environ. Microbiol. **33**:1141–1148.
3. **Bagley, S. T., and R. J. Seidler.** 1978. Primary *Klebsiella* identification with MacConkey-inositol-carbenicillin agar. Appl. Environ. Microbiol. **36**:536–538.
4. **Bagley, S. T., R. J. Seidler, and D. J. Brenner.** 1981. *Klebsiella planticola* sp. nov.: a new species of *Enterobacteriaceae* found primarily in non clinical environments. Curr. Microbiol. **6**:105–109.
5. **Berry, J. M., L. D. Witter, and J. F. Folinazzo.** 1956. Growth characteristics of spoilage organisms in orange juice and concentrate. Food Technol. **10**:553–556.
6. **Biamon, E. J., and T. C. Hazen.** 1983. Survival and distribution of *Aeromonas hydrophila* in near-shore coastal waters of Puerto Rico receiving rum distillery effluent. Water Res. **17**:319–326.
7. **Caplenas, N. R., M. S. Kanarek, and A. P. Dufour.** 1981. Source and extent of *Klebsiella pneumoniae* in the paper industry. Appl. Environ. Microbiol. **42**:779–785.
8. **Duncan, D. W., and W. E. Razzell.** 1972. *Klebsiella* biotypes among coliforms isolated from forest environments and farm produce. Appl. Microbiol. **24**:933–938.
9. **Frazier, W. C., and D. C. Westhoff.** 1978. Food microbiology. McGraw-Hill Book Co., New York.
10. **Harris, R. H., and R. Mitchell.** 1973. The role of polymers in microbial aggregation. Annu. Rev. Microbiol. **27**:46–73.
11. **Huntley, B. F., A. C. Jones, and V. J. Cabelli.** 1976. *Klebsiella* densities in waters receiving wood pulp effluents. J. Water Pollut. Control Fed. **48**:1766–1771.
12. **Jiwa, S. F., H. Krovacek, and T. Wadström.** 1981. Enterotoxigenic bacteria in food and water from an Ethiopian community. Appl. Environ. Microbiol. **41**:1010–1019.
13. **Knittel, M. D., R. J. Seidler, C. Eby, and L. M. Cabe.** 1977. Colonization of the botanical environment by *Klebsiella* isolates of pathogenic origin. Appl. Environ. Microbiol. **34**:557–563.
14. **Mundt, J. O., J. L. Collins, I. E. McCarthy, and R. Barley.** 1978. Description and microbiology of home-canned tomatoes and tomato juice. J. Food Prot. **41**:944–947.
15. **Mundt, J. O., and M. A. Daeschel.** 1979. Survival of *Klebsiella pneumoniae* heated in buffer and in tomato juice. J. Food Prot. **42**:933–935.
16. **Murdock, D. I., and W. S. Hatcher.** 1975. Growth of microorganisms in chilled orange juice. J. Milk Food Technol. **38**:393–396.
17. **Niemelä, S. I., and P. Väätänem.** 1982. Survival in lake water of *Klebsiella pneumoniae* discharged by a paper mill. Appl. Environ. Microbiol. **44**:264–269.
18. **Sjogren, R. E., and M. J. Gibson.** 1981. Bacterial survival in a dilute environment. Appl. Environ. Microbiol. **41**:1331–1336.
19. **Vasconcelos, G. J., and R. G. Swartz.** 1976. Survival of bacteria in seawater using a diffusion chamber apparatus in situ. Appl. Environ. Microbiol. **31**:913–920.
20. **Wilkinson, J. F.** 1958. The extracellular polysaccharides of bacteria. Bacteriol. Rev. **22**:46–73.