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SURVIVAL OF *YERSINIA ENTERECOLITICA* AND SOME *ENTEROBACTERIACEAE* SPECIES IN YOGHURT

YERSINIA ENTEROCOLITICA VE BAZI *ENTEROBACTERIACEAE* TÜRLERİNİN YOĞURTTA CANLI KALMA DÜZEYLERİ

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ÖZET: Bu çalışmada *Yersinia enterocolitica, Klebsiella pneumonia, Escherichia coli* ve *Enterobacter aerogenes*'in yoğurt yapımı ve depolanması sırasında canlı kalma düzeyleri incelenmiştir. Çalışmada elde edilen sonuçlar, tüm bakteri türlerinin gerek üretim gerekse depolanmaları sırasında inaktivasyona uğradığını ve inaktivasyon oranının başlangıçtaki inokulum düzeyi ile yoğurdun pH değerindeki azalmaya bağlı olduğunu göstermiştir.

SUMMARY: In this study, the survival of *Y. enterocolitica, K. pneumonia, E. coli* and *E. aerogenes* in yoghurt prepared from cow's milk was examined.

The results of this study showed that all bacteria species are destroyed in yoghurt and the rate of destruction depends on the size of the initial inoculum and the rate of pH value reducing.

INTRODUCTION

Milk is suitable for the growth bacterial species including pathogens. They may contaminate to milk either directly from cow or from the milk handler, and certainly unsuitable dairy manufacturing processes. So dairy products are still caused occasional outbreaks of disease and food poisonings.

The bacteria including pathogens has been isolated and identified from many food stuffs such as beef, icecream, sea food, milk and milk products, etc (GUTMAN et al. 1973, LEISTNER et al. 1975, LEE 1977, SCHIEMANN 1980, MANTIS et al 1982, MORSE et al. 1984). For this reason especially raw milk and other dairy products were been generally found contaminated with *Yersinia enterocolitica, Esherichia coli, Klebsiella pneumonia, Enterobacter aerogenes* (SCHIEMANN and TOMA 1978, SCHIEMANN 1978, BARETT 1986).

Yersinia enterocolitica and other Enterobacters produces highly more clinical syndromes in humans. Particularly acute gastroenteritis or enterocolitis is a frequent clinical syndromes of the infection and certainly contaminated foods play a main role in the way of transmission. On the other hand a variety of other illness may eventually result from especially *Y. enterocolitica* contamination. These include painful swellings and blotches mainly on legs and arthritis, or polyarthritis and septic anaemia (BOTTONE 1977, VANDEPITTE and WAUTERS 1979, MARKS et al. 1980, KAPPERUD and BERGAN 1984).

So the presence of *Y. enterocolitica* and other *Enterobacter*'s in raw milk is increased the potential risk of transmission by varied milk products that are produced from raw milk and also contaminated during processing. In the present work the survival of *Y. enterocolitica* and other *Enterobacter*'s during manufacturing and storage period are examined.

MATERIAL and METHODS

Material

Milk: Pasteurized cow's milk has been used.

Lactic culture: Liquid yoghurt culture was used that supplied from Aegean University Agricultural Faculty Milk Technology Department was used. In the study, bulk culture was prepared in sterile skim milk the day before yogurt was made.

***Yersinia enterocolitica*:** Strain 0:9 (supplied by the Pasteur Institute Paris) was used. The strain was kept on Tripticase soy agar slants. Inoculum was prepared after two passage 18 h fresh cultures in Selenite cystine broth at 22^0C and inoculated in yoghurt milk at the rate of 10^4-10^5 or/ml (KENDALL and GILBERT 1980, MANTIS et al. 1982).

Klebsiella pneumonia: *K. pneumonia* CCM 2318 was used. The strain was preserved in Nutrient agar slants. Inoculum (10^5-10^6 or/g) was prepared after two passage in Nutrient broth at 30,1±2°C.

Esherichia coli and ***Enterobacter aerogenes:*** *E. coli* ATTC 11230 and *E. aerogenes* CCM 4310 were used. The strains were kept on Nutrient agar slants. Both inoculum were prepared after two passage 18 h fresh cultures in Nutrient broth at 30±2°C and inoculated in yoghurt milk at the rate of 10^5-10^6 or/ml.

Yoghurt preparation: The yoghurt milk was heated in a water bath at 90°C for 10 minutes, cooled at 45°C and the starter culture (2% v/v) was added. Then *Y. enterocolitica, K. pneumonia, E.coli* and *E. aerogenes* culture inoculated separately and incubated at 42±2°C until forming a firm coagulum. After coagulation yoghurt samples were stored at +5±1°C for 3 days.

Methods

Enumeration of *Y.enterocolitica*: The enumeration of *Y.enterocolitica* in yoghurt was performed by surface plating on plates containing Mc Conkey agar.Buffered phosphate diluent was used as dilution. The plates were incubated at 22°C for 48 h (FEELEY et al. 1976, ANONYMOUS 1978) and finally having translucent pink colour center *Yersinia* colonies were counted.

Enumeration of *K.pneumonia*: The enumeration of *K.pneumonia* in yoghurt carried out by pour plate method on plates containing Tergitol 7 agar. The plates were incubated at 37 2°C up to 24 hours. After the incubation typical colonies (greenish, yellow large mucoid) were counted (ANONYMOUS 1987).

Y. enterocolitica, K.pneumonia, E.coli and *E.aerogenes* are given in Table 1.

Table 1. Survival of *Y.enterocolitica, K.pneumonia, E.coli* and *E.aerogenes* During The Storage of Yoghurt at 5±1°C (or/ml).

Time after	Temperature	pH	*Y.enterocolitica*	*K.pneumonia*	*E.coli*	*E.aerogenes*
0 h	42°C	6.65	$9.7x10^5$	$6.0x10^6$	$6.4x10^6$	$6.6x10^6$
4 h	42°C	4.70	$1.2x10^6$	$4.9x10^5$	$4.0x10^5$	$3.7x10^5$
12 h	5°C	4.60	$1.6x10^4$	$9.8x10^1$	$2.4x10^4$	$2.1x10^4$
24 h	5°C	4.50	$1.1x10^3$	$4.7x10^3$	$1.8x10^3$	$1.9x10^3$
36 h	5°C	4.40	$1.8x10^2$	$2.8x10^2$	$3.9x10^2$	$1.9x10^2$
48 h	5°C	4.25	84	34	-	-
3 days	5°C	4.10	-	-	-	-

The results of experiments given in Table it showed that all bacteria species except *Y.enterocolitica* decreased during the initial hours of incubation, whereas *Y.enterocolitica* colonies stayed almost the same. In addition, it is evident, that the bacteria cultures used in the experiments have survived up to 36 hours. *Y.enterocolitica* colonies ranging between $9.7x10^5$ or/ml and $1.2x10^6$ or/ml during the fermentation were reduced to less & 84 or/ml with in 48 hours storage.

At the same time, the other bacteria cultures have been more rapidly than *Y.enterocolitica* cells. The initial inoculum size of *K.pneumonia* $6,0x10^6$ or/ml have been reduced & 34 or/ml in 48 hours also. On the other hand, the initial enumeration of *E.coli* and *E.aerogenes* have rapidly decreased from $6,4x10^6$ and $6,6x10^6$ or/ml to $3,9x10^2$ and $1,9x10^2$ or/ml respectively.

According to the results, all bacteria species including *Y.enterocolitica* became inactive and finally died in yoghurt. But it can be stated that the rate of inactivation seems to be slower than the other Gram negative bacteria (GOEL et al. 1971). But in contrary, the results of the previously done researches (HANNA et al. 1977) revealed that *Y.enterocolitica* and Gram negative bacteria under same conditions can grow slowly at pH values above 4.6. Although the decreasing rates of bacteria cultures found in our study were low, but because the acidity develops comparatively fast, *Y.enterocolitica* and other cultures were inactivated within 48 h depending on the size of inoculum rate and production method (MANTIS and KILIKIDIS 1972, MANTIS et al. 1982).

As a result of this study, it can be pointed out that a recontamination of milk with *Y.Enterocolitica, K.Pneumonia, E.Coli* and *E.aerogenes* after its treatment may be hazardous for consumer. In addition, the

acid environments of yoghurt in the inactivation rate of the bacteria is not the only effective factor and should not be considered alone.

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