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Effect of the Suspending Medium on the Growth and Susceptibility to Heat Stress of *Yersinia enterocolitica*

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Abstract. *Yersinia enterocolitica* cells incubated at 4 and 25°C were able to grow in chicken and in beef ground meats, whereas they only retained viability in chocolate milk, milk or water. Heating cells at 48°C for 30 min in the ground meats of chicken and beef, in chocolate milk and in milk did not cause any heat injury to them, whereas, the number of viable cells decreased at the same temperature in water or buffer.

Introduction

Yersinia enterocolitica is a gram-negative rod of the family Enterobacteriaceae [1, pp. 448-506] and is a causative agent in single cases and mass outbreaks of human yersiniosis in many countries throughout the world [2]. Many of the virulence traits of this organism are known to be coded by a 70 Kilobase plasmid [3,4].

Typical strains of *Y. enterocolitica* have been isolated from drinking and surface waters in Norway, Canada and the United States [5-10] and from contaminated foods such as milk, milk products and meat [11-13]. The bacterium has the ability to grow at temperatures approaching 0°C and this ability has been used as the basis for its isolation from many natural sources [14-16].

As a result of the high incidences of *Y. enterocolitica* infection in man, this preliminary study was undertaken to evaluate the ability of this bacterium to grow and/or survive in meat, milk and water incubated at two different temperatures. Further, the effect of heat stress on cells inoculated into the above naturally-occurring media was evaluated.

Materials and Methods

Organism

Y. enterocolitica ATCC 23715 was used. Stock cultures were stored in 1% peptone water containing 50% glycerol at −20°C.

Media

The peptone-D-sorbitol medium of Weagant and Kaysner [17] was used as a non-selective growth medium (medium A) or as a selective growth medium (medium B) by the addition of 0.15% bile salts. For a solid medium 1.5% agar was added to medium A or B.

Natural Samples

Chicken and beef ground meats (tender meat; without bones or skin) were obtained from a local grocery store immediately after slaughter. Cow's milk and chocolate milk (percentage composition of chocolate milk: lactose 4, protein 3, butter fat 2, vitamin D 400 i.u. and chocolate) were obtained from a local supermarket. Chocolate milk was implicated in *Y. enterocolitica* infections [18], therefore, it is included in this study. The drinking water was obtained from the water supply of Riyadh. The ground meats and the drinking water were sterilized by autoclaving. The milk and chocolate milk were all ultra high temperature treated, therefore, they were used directly under sterilized conditions.

Growth at 4 and 25°C

The ability of *Y. enterocolitica* to grow in the above natural samples was studied. *Y. enterocolitica* cells were grown in medium A to the middle of the exponential phase, washed with and suspended in 0.87% NaCl. The cell suspension was used to inoculate six sets of each sample to a final cell concentration of $\approx 10^4$ cells/gram or/ml as estimated by diluting with 0.87% NaCl and plating on medium A. Three sets of the inoculated tubes were incubated at 4°C and the other three sets were incubated at 25°C. Samples were removed at zero time and at 24hr intervals for a period of 6 days, diluted with 0.87% saline and plated on medium A. The colonies were counted ater 24hr incubation at 37°C. The log number of the average colony-forming units was drawn versus time.

Heat Stress

The above saline cell suspensions were used to inoculate three sterile sets of tubes of each of chicken ground meat, beef ground meat, chocolate milk, milk, water and phosphate buffer to a final cell concentration of $\approx 10^8$ cells/gram or/ml. The tubes were placed in a 48°C water bath. Samples were removed for enumeration at zero time and at 15 and 30 min thereafter. Appropriate dilutions were made with

0.87% NaCl and spread on medium A and on medium B. The colonies were counted after 24 hr incubation at 37°C. The log number of the average colony-forming units was drawn versus time.

Results

Growth at 4 and 25°C

The growth ability of *Y. enterocolitica* in ground meats of chicken and beef. chocolate milk, milk and water samples incubated at 4 and 25°C is shown in Fig. 1 (A-E), respectively. *Y. enterocolitica* was able to grow in the ground meats of chicken and beef at both temperatures, their growth, however, at 25°C was better than that at 4°C. Further, cells incubated at 25°C grew better in ground chicken than in ground beef. A minor or no increase was seen for cells inoculated into chocolate milk, milk and water. Further, the growth curves for those cells incubated at 4°C and for those cells incubated at 25°C were similar, but there was a longer lag period at 4°C. The ground meats of chicken and beef appear to support the growth of *Y. enterocolitica* at 4 and 25°C, whereas, chocolate milk, milk and water appear to retain viability only. Water, however, did not retain viability to the same extent as the milk media.

Heat Stress

The effect of heat stress on the viability of *Y. enterocolitica* and on its ability to grow in the presence of a selective agent (bile salts) is shown in Fig. 2 (A-F). The bile salts was used as an indicator of heat stress. The outer membrane of the heat stressed cells can be damaged, so the cells become permeable and accordingly sensitive to bile salts. Cells which were heat stressed in the ground meats of chicken and beef and in chocolate milk and milk were able to resist heat. This heat resistance was indicated by the retention of viability to a similar extent in the selective medium (medium B) and in the nonselective medium (medium A). Cells which were heat stressed in water or phosphate buffer, however, were heat injured as indicated by the decrease in their number with time and by their sensitivity to bile salts present in medium B. This sensitivity was concluded from the greater decrease of the number of cells plated on the selective medium as compared with those plated on the nonselective medium. The response to the heat stress, therefore, depends on the nature of the environment.

Discussion

Yersiniosis is regarded as a food or water borne disease because of the implication of food or water [5-10] in several outbreaks. *Y. enterocolitica* is known to grow in some foods incubated at 37°C [19]. However, it is not known if the growth ability of this organism in such foods changes with temperature. In general, our results have shown that *Y. enterocolitica* cells incubated at 4 and 25°C expressed the same

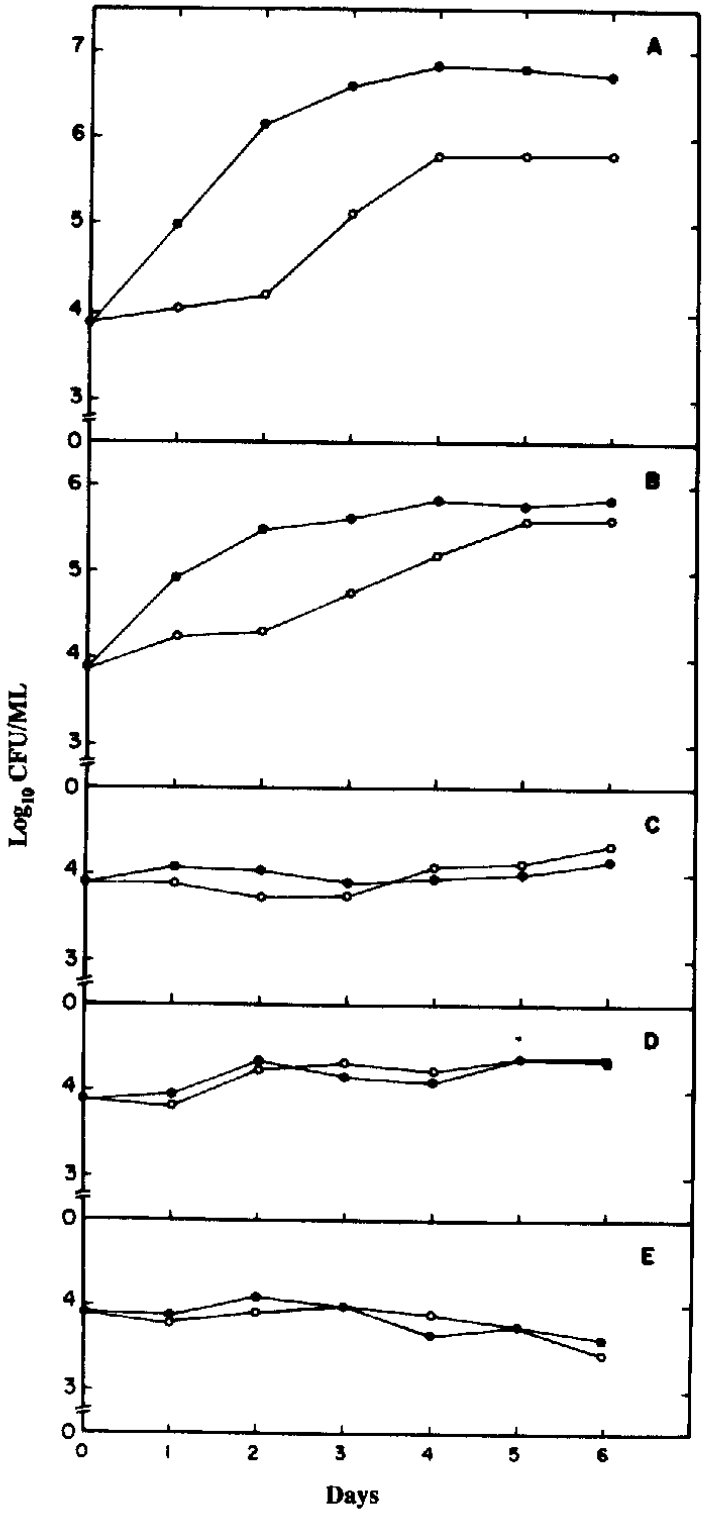

Fig. 1. Growth of *Y. enterocolitica* in: Chicken ground meat (A) beef ground meat, (B) chocolate milk, (C) milk (D) and water and (E) open symbols represent cells incubated at 4°C. Solid symbols represent cells incubated at 25°C.

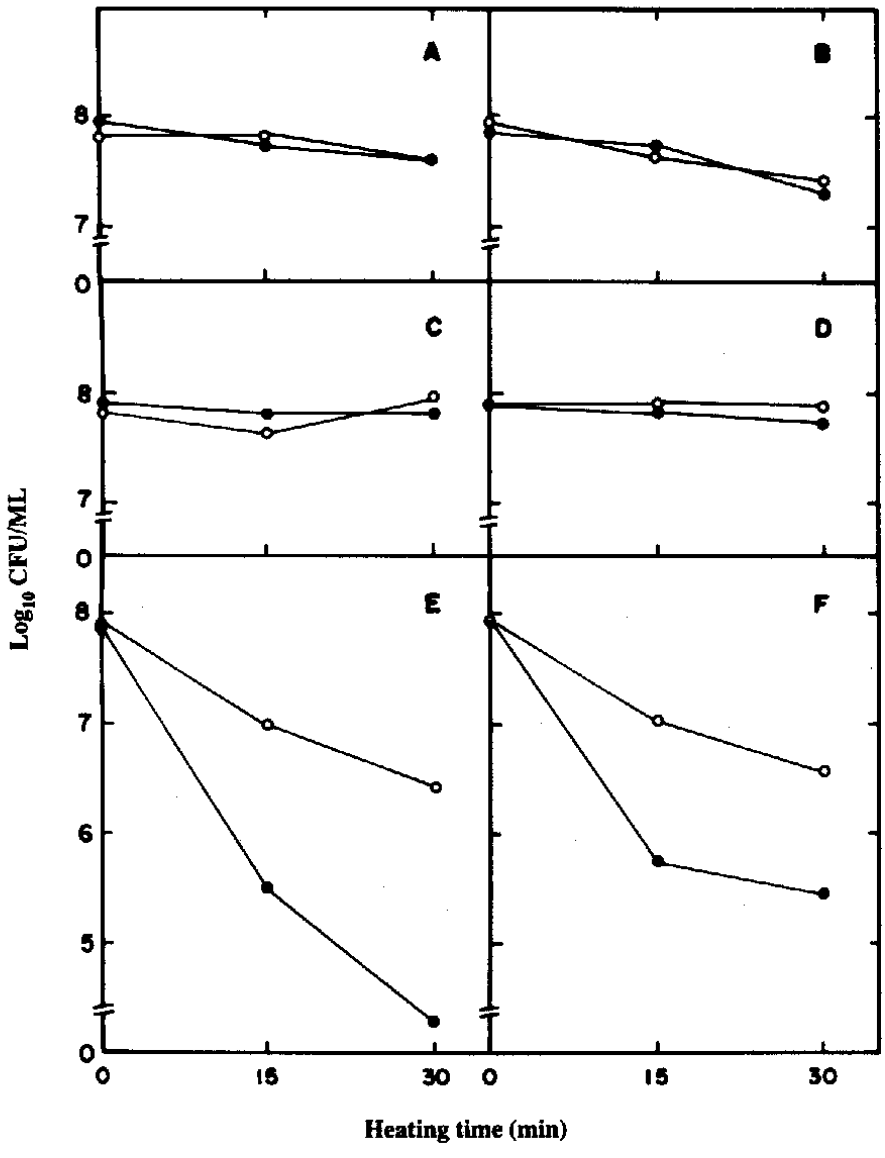

Fig. 2. Survival curves for cells of *Y. enterocolitica* heated at 48°C in: chicken ground meat (A) beef ground meat, (B) chocolate milk, (C) milk, (D) water, (E) buffer and (F) Cells were plated at times indicated on non-selective medium (open symbols) and on selective medium (solid symbols)

behavior independent of the growth temperature. That is their number increased in meat, but did not increase in chocolate milk, milk and water, eventhough they remained viable. This fact might explain the greater recovery of this organism from meats [13,20,21]. Further, our results, showed that *Y. enterocolitica* cells present in chocolate milk, milk and water could remain viable and can cause great threat to humans if exposed to temperatures higher than 30°C, because the cells will not only remain viable but also will increase in number.

A minor decrease was shown for those cells which were inoculated into water after 5 days incubation at both temperatures, this decrease might be due to the lysis of such cells. The lysis, however, was prevented when the cells were present in a more osmotically stable environment such as chocolate milk and milk.

Temperatures greater than the optimum are well known to cause injury to bacteria [22], this injury would be of importance in a country such as Saudi Arabia where the summer temperature reaches 48°C. A preliminary study, therefore, was performed to evaluate the effect of this temperature on *Y. enterocolitica* cells present in meat, in chocolate milk, in milk, in water and in buffer. This preliminary study will assist us in a study to be carried out later during the summer season for the isolation of this bacterium from similar natural environments.

Injured bacteria are well known to be susceptible to bile salts that are present in the selective media, because of the damage of their outer membrane [23]. In our study, accordingly, little or no injury was seen for those cells heated in meat, chocolate milk and milk. The above findings were based on the slope of the obtained curves and on the resistance to the selective agent under study. Injury, however, was noticeable for those cells heat-stressed in water or buffer as estimated from the decrease in the number of cells with time and the poorer recovery of cells plated on the selective medium as compared with those plated on the non-selective medium. The osmotically stable environment present in meats, chocolate milk and milk presumably protects the cells from the injury which occurs in the water and in the butter.

In summary, the ability of *Y. enterocolitica* to grow at 4 and 25°C depends on the nature of the environment in which they are present. Further, the nature of the environment determines the degree of injury to which they are susceptible after heat-stress.

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تأثير بيئة المعلق على النمو والإجهاد الحراري للبكتيريا يرسينيا انتيروكوليتيكا

علي عبدالله السلامة و شريف عزت مكي

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(استلم في ٨ رجب ١٤١٠هـ، قُبل للنشر في ١٨ ربيع الأول ١٤١١هـ)

ملخص البحث. لقد نمت خلايا البكتيريا يرسينيا انتيروكوليتيكا في لحم الدجاج أو في لحم الأبقار المفروم والمحضن عند درجتي حرارة ٤ و٢٥°م. بينما احتفظت بحيويتها فقط في حليب الشكولاتة، الحليب، الماء المحضنين عند درجتي الحرارة نفسيهما. لم تجهد حراريًا خلايا هذه البكتيريا الملقحة في لحم الدجاج أو لحم الأبقار المفروم، حليب الشكولاتة، الحليب، وذلك بعد التعريض لدرجة حرارة ٤٨°م لمدة ٣٠ دقيقة، بينما نقصت أعداد الخلايا الحية الملقحة في الماء أو المحلول المنظم بعد التعريض أعلاه.