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Effect of Enrichment Media, Temperature and Time of Enrichment on *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

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Abstract. The growth characteristics of *Yersinia enterocolitica* and *Y. pseudotuberculosis* under selective conditions of media, temperature and time were compared. Strains of *Y. enterocolitica* maintained their viable count up to 35 days in enrichment broth upon incubation at 4°C and showed variable amount of increase and time of decline upon incubation at 26°C. Strains of *Y. pseudotuberculosis* that were inoculated into enrichment broth maintained their viable count up to 14 days upon incubation at 4°C, and decreased sharply upon incubation at 26°C. Strain NCTC 10460 of *Y. enterocolitica* and both strains of *Y. pseudotuberculosis* that were inoculated into phosphate-buffered saline increased and maintained their viable count up to the end of the experiment (63 days), whereas, strain ATCC 23715 of *Y. enterocolitica* declined with time. Accordingly,enrichment in phosphate-bufferd saline at 4°C could be used if enriching for the two species is aimed. If enriching for one species, however, phosphate-buffered saline (4°C) should be used for *Y. pseudotuberculosis* and Yersinia enrichment broth (26°C) for *Y. enterocolitica*.

Introduction

Yersinia enterocolitica and *Y. pseudotuberculosis* are organisms that have been receiving increasing attention as an important cause of food and water-borne illness such as gastroenteritis and terminal illitis [1-4]. Strains from both species have been isolated from different parts of the world, including the United States [5;6], Saudi Arabia [7], Japan [8-12], France [13], United Kingdom [3], Zaire [14], and Australia [15].

In pure cultures strains of *Y. enterocolitica* and *Y. pseudotuberculosis* grow well on a variety of media such as blood, brain heart infusion and MacConkey agars. However, recovery of these organisms from environmental and clinical samples requires some selective conditions. The above two species are among the few enteric pathogens capable of growth at refrigeration temperature (4°C). Samples to be analyzed for the presence of these two species, therefore, are usually incubated at 4°C for 2 to 4 weeks, depending on the initial contamination level of the samples to be analyzed and the type of enrichment broth used.

The present investigation was carried out to evaluate further the effect of the length of enrichment period, temperature of enrichment, enrichment and plating media on the viable counts of the two bacterial species under study.

Materials and Methods

Yersinia strains

Two strains of *Yersinia pseudotuberculosis* and two strains of *Yersinia enterocolitica* were used in this study (Table 1). Stock cultures were kept as cell suspensions at − 20°C in 30% glycerol-1 % phosphate.

Table 1. Yersinia strains used

Organism	Strain	Source
Y. enterocolitica	ATCC 23715	American Type Culture Collection
	NCTC 10460	National Collection of Type Cultures
Y. pseudotuberculosis	NCTC 10275	National Collection of Type Cultures
	NCTC 827	National Collection of Type Cultures

Enrichment media

Standard enrichment media which have been described previously [7;16] and selected for comparative purposes in this study include: phosphate buffered saline [PH 7.6) and Yersinia enrichment broth (Merck). Media were prepared in 50 ml quantities using 250 ml flasks. One set was prepared from phosphate buffered saline and incubated at 4°C. Two sets were prepared from Yersinia enrichment broth, one set was inclubated at 4°C, the other was incubated at 26°C. Each set of flasks was inoculated with the four Yersinia strains under study; one strain per flask. Samples were removed weekly, diluted with 0.85% saline, spread on plates of blood agar base

(Merck) and Yersinia selective agar (Merck). The colonies were counted after 48 h incubation at 26°C and the log. number of cells was drawn versus time. Each experiment has been replicated three times and three replicates were taken at each time point.

Results

Growth characteristic of *Yersinia enterocolitica.*

The growth curves of *Y. enterocolitica* strains ATCC 23715 and NCTC 10460 are shown in Figs 1 and 2, respectively. Cells of both strains that were inoculated into Yersinia selective broth and incubated at 4°C, maintained their numbers until day 35 and then began to decline, whereas, those incubated at 26°C behaved differently, that is, Strain ATCC increased one log. and began to decline after 7 days and strain

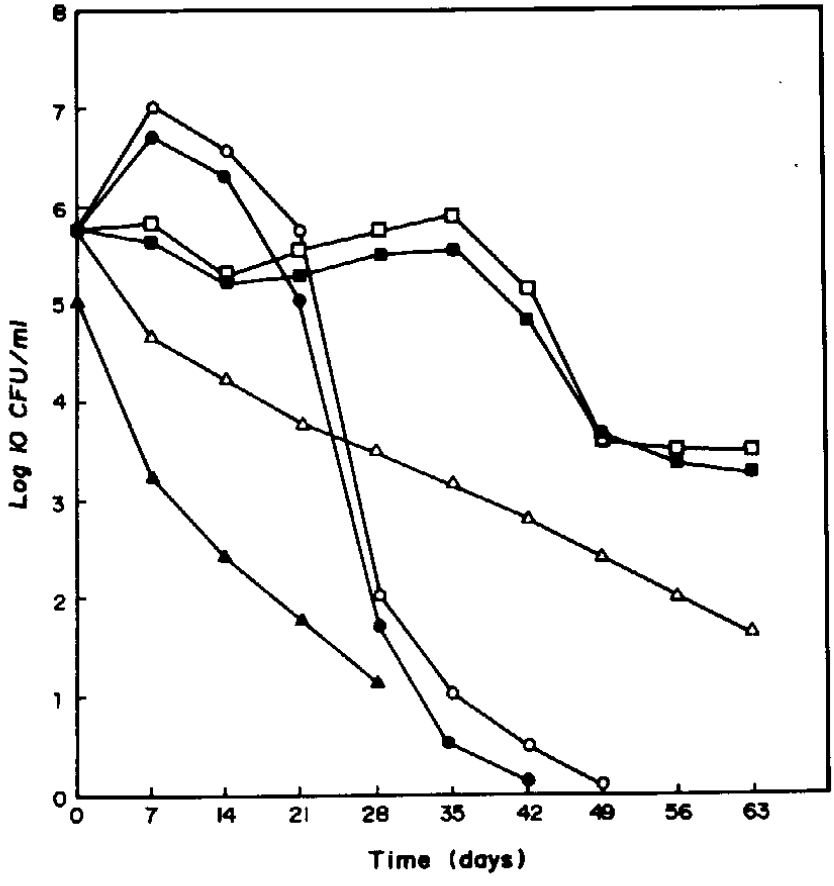

Fig. 1. Growth curves of *Y. enterocolitica* ATCC 23715. Open symbols represent cells plated on blood agar base, closed symbols represent cells plated on Yersinia selective agar. Squares and circles represent cells inoculated into yersinia enrichment broth and incubated at 4°C and 26°C, respectively. Triangles, represent cells inoculated into phosphate buffered saline and incubated at 4°C.

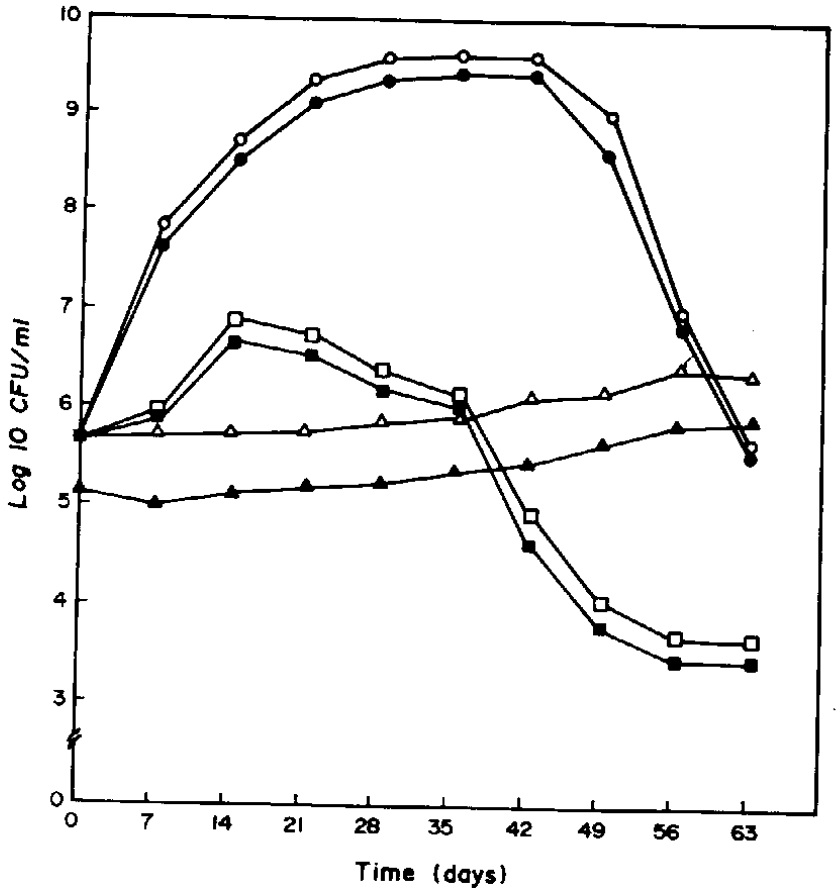

Fig. 2. Growth curves of *Y. enterocolitica* NCTC 10460. See legend to Fig. 1 for symbols.

NCTC 10460 increased four logs. and began to decline after 42 days. Cells that were inoculated into phosphate-buffered saline and incubated at 4°C behaved differently, that is, the viable count of strain ATCC 23715 declined, whereas, the viable count of strain NCTC 10460 maintained with some increase up to the end of the experiment (day 63).

Growth characteristic of *Yersinia pseudotuberculosis*.

The growth curves of *Y. pseudotuberculosis* are shown in Figs 3 and 4, respectively. Cells of both strains that were inoculated into Yersinia selective broth and incubated at 4°C maintained their numbers to some extent until day 14, then decreased gradually, whereas, those incubated at 26°C decreased sharply. Cells that were inoculated into phosphate-buffered saline and incubated at 4°C behaved similarly to some extent. They first decreased, then increased and maintained their numbers up to the end of the experiment (day 63).

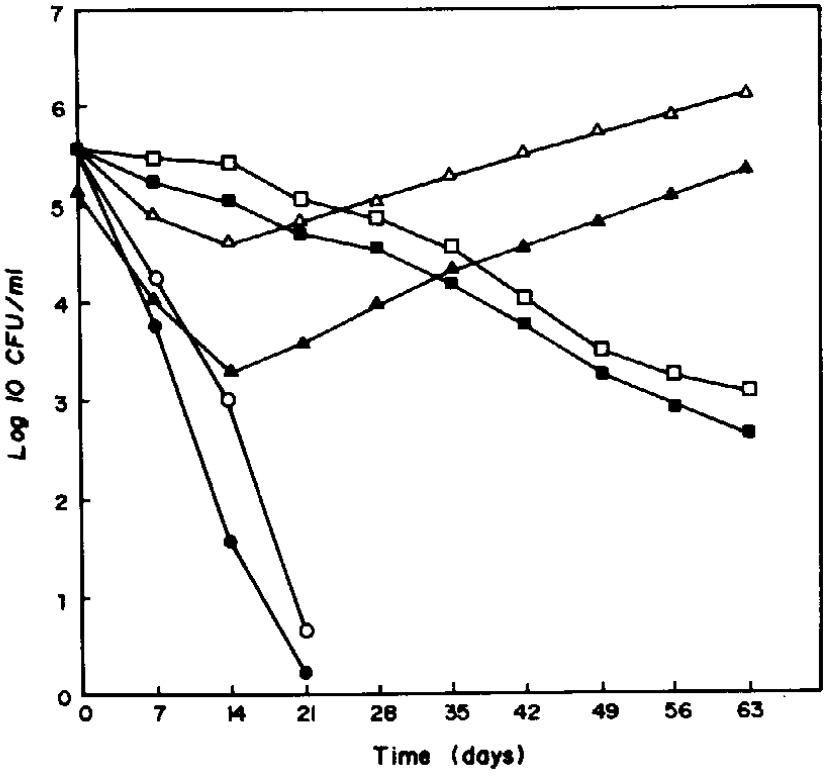

Fig. 3. Growth curves of *Y. pseudotuberculosis* NCTC 10275. See legend to Fig. 1 for symbols.

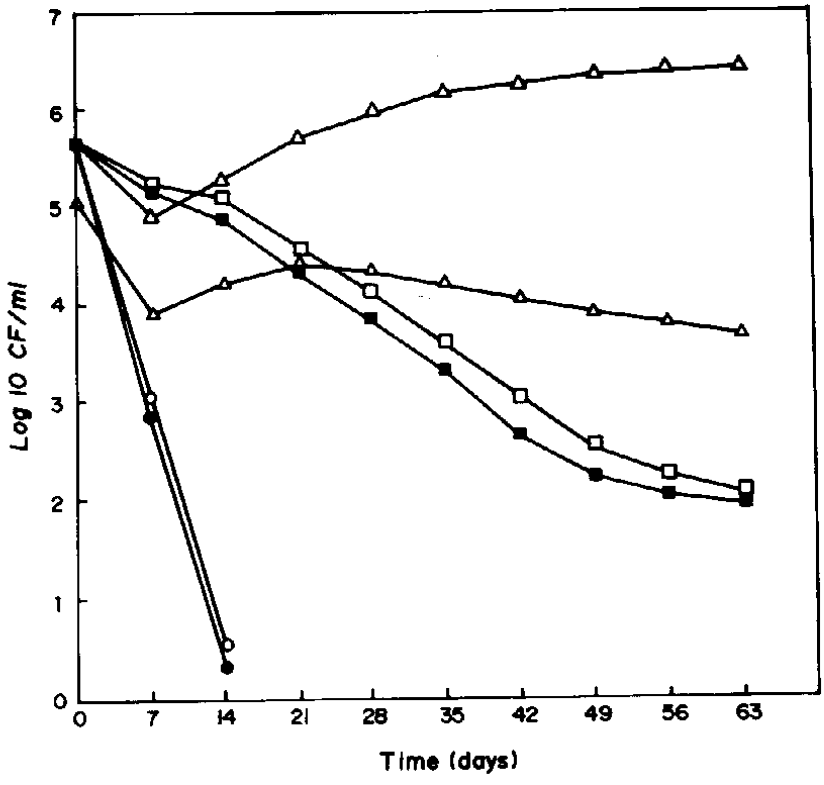

Fig. 4. Growth curves of *Y. pseudotuberculosis* NCTC 827. See legend to Fig. 1 for symbols.

For both Yersinia strains under study, the viable count obtained by plating on blood agar base was always higher than the viable count obtained by plating on Yersinia selective agar, particularly, for cells inoculated into phosphate-buffered saline.

Discussion

In clinical samples, Yersinia is always present with other bacteria. It is expected, therefore, from the enrichment media to allow Yersinia strains to increase or at least to maintain their numbers and the accompanying bacteria to fail to grow or decline in number. The results of this study show that the Yersinia enrichment broth incubated at 26°C was not appropriate for the isolation of *Y. pseudotuberculosis,* because the viable count of both of its two strains under study declined rapidly; whereas, it is appropriate for the isolation of *Y. enterocolitica* with an average incubation time of 7 days for the two strains under study. Yersinia enrichment broth incubated at 4°C is not appropriate for the isolation of both species, because no increase in the viable count for both *Y. enterocolitica* strains was noted, whereas, both *Y. pseudotuberculosis* strains decreased in number.

The usual enrichment time for *Y. enterocolitica* and *Y. pseudotuberculosis* in phosphate buffered saline incubated at 4°C, is 21 days as has been reported by many authors [11; 16-18]. From the study reported herein it seems that enrichment in phosphate buffered saline is more appropriate for both strains of *Y. pseudotuberculosis* which increased in number compard to *Y. enterocolitica* where one of its strains increased and the other strain (ATCC 23715) decreased. However, since the contaminating bacteria decline faster than strain ATCC 23715, selection in phosphate-buffered saline at 4 °C seems to be the best general method to be used for both *Yersinia* species under study. If enriching for one species, however, it is advisable to enrich for *Y. pseudotuberculosis* in phosphate-buffered saline with an incubation at 4°C for not less than 21 days, and enrich for *Y. enterocolitica* in Yersinia selective broth incubated at 26°C for 14 days. It is preferable that plating after phsophate-buffered saline enrichment should be in a non-selective medium, because the phosphate-buffer enriched Yersinia cells are, unlike those enriched in Yersinia selective broth, sensitive to the selective agents present in the Yersinia selective agar, that is cells enriched in Yersinia selective broth are more tolerant to the selective agents present in the Yersinia selective agar.

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تأثير بيئة الإكثار ودرجة حرارته ومدته على البكتريا *يرسينيا انتيروكوليتيكا* و*يرسينيا بسيودوتيوبركيولوسس*

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(سُلِّمَ في ١٤١٤/١٢/١هـ؛ وقُبل للنشر في ١٤١٥/٦/٢٦هـ).

ملخص البحث. تمّت مقارنة صفة النمو للبكتريا *يرسينيا انتيروكوليتيكا* و*يرسينيا بسيودوتيوبركيولوسس* وذلك تحت ظروف انتخابية من بيئة النمو والحرارة والوقت. سلالات *يرسينيا انتيروكوليتيكا* المنمّاة في المرق المغذي الانتخابي احتفظت بعددها الحي لمدة تصل إلى ٣٥ يومًا في حالة تحضينها عند ٤°م، ولكنها أبدت كميّات متغايرة من الزيادة ووقت التناقص في حالة تحضينها عند ٢٦°م. سلالات *يرسينيا بسيودوتيوبركيولوسس* المنمّاة في المرق المغذي الانتخابي احتفظت بعددها الحي لمدة تصل إلى ١٤ يومًا أثناء تحضينها عند ٤°م وتناقصت بشكل كبير أثناء تحضينها عند ٢٦°م. السلالة رقم NCTC 10460 *ليرسينيا انتيروكوليتيكا* وكِلا سلالتي *يرسينيا بسيودوتيوبركيولوسس* التي نمت في محلول الملح المنظم بالفوسفات ازدادت واحتفظت بعددها الحي حتى نهاية التجربة (٦٣ يوما)، في حين أن سلالة ATCC 23715 *ليرسينيا انتيروكوليتيكا* تناقص عددها مع مرور الوقت.

بناءً على ما سبق، يُنْصح باستخدام المحلول الملحي المنظم بالفوسفات عند ٤°م في حالة الرغبة بعزل كِلا النوعين، أما في حالة الرغبة بعزل نوع واحد فقط فينصح باستخدام المحلول الملحي المنظم بالفوسفات عند ٤°م للحصول على *يرسينيا بسيودوتيوبركيولوسس* واستخدام المرق المغذي الانتخابي عند ٢٦°م للحصول على *يرسينيا انتيروكوليتيكا*.