The Yersinia enterocolitica species tolerance to temperature

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Abstract

The behaviour of Yersinia enterocolitica's species to different temperatures has been studied. The tests have been carried out on artificial contaminated food products and liquid or solid mediums inoculated with purified strains. Following the contamination sources represented by refrigerated food and cocked food, we have considered appropriate to carry out some investigations that revealed the optimum growing and preserving temperatures of Yersinia enterocolitica, their life period at low temperatures (frozen or refrigerated food products) or at high temperatures (boiled food products).

The analysis of the results obtained has demonstrated that Yersinia enterocolitica species are not completely destroyed by freezing temperatures (at –18 and –12℃ in max. 20 days), but are destroyed relatively quickly at high temperatures (at 70℃ in max. 10 min.). Instead, the refrigerating temperature or the temperatures between 10 to 40℃ offer the preservation or multiplication conditions for Yersinia enterocolitica strains.

Keywords: Yersinia enterocolitica, tolerance, temperature

The current interest posed by the *Yersinia* species is determined by the fact that these bacterial species generate a series of morbid entities which come more and more frequent to the attention of bacteriologists, epidemiologists and surgeons. Numerous studies have been published regarding the infections caused by these bacteria's during the latest years, and the researches on this theme developed. During the last ten years of the last millennium, numerous researchers drew the attention on the fact that the bacteria species of Yersinia are frequently involved in the food borne diseases with acute diarrheic syndrome in humans (3, 10, 13, 15).

Although clinically these *Yersinia* infections do not determine great complications and are generally efficient treated, the epidemiological risk is important, outbreaks with hundreds of cases being noticed (3, 8, 11, 12). First, *Yersinia pestis* was considered as posing the most important epidemiological risk (1, 3). Further on, many research teams have discovered that many species are involved in food borne diseases of humans (1, 4, 7, 8), some of them causing different morbid entities in different animal species (11). Currently, although many *Yersinia* species have been discovered and taxonomically classified, three species are considered as the main pathogens for humans: *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. Having in view that the contamination sources for humans are generally represented by unprocessed food or by food that had in the processing technology short periods of heat treatment and of maximum 70 °C, we have considered appropriate to carry out investigations that would reveal the optimum preservation and growing temperatures of *Yersinia enterocolitica* strains, the survival period of these bacteria species at low temperatures (in refrigerated or frozen food) or at high temperatures (in heat treated food).

Materials and Methods

The behaviors of different *Yersinia enterocolitica* strains at different temperatures have been studied. The tests have been carried out both on artificial contaminated food products as well as on liquid or solid medium inoculated with purified strains.

The analyzed bacteria strains in this study have been isolated from different food samples or faeces samples taken from human subjects with acute diarrheic syndrome, identified by comparative biochemical tests and named with code numbers. The bacteria strains in the study are represented by: Y. e. 1025pa (isolated from foods with aquatic origin); Y. e. 2732c and 1432c (isolated from pig minced meat); Y. e. 816b and 2696b (isolated from bovine minced meat); Y. e.1946p and 2682p (isolated from poultry meat); Y. e. 1765pl, 3788pl and 3781pl (isolated from raw milk).

In the first stage, young cultures (of maximum 24 hours) have been obtained from purified strains, cultures that have been used either for artificial contamination of food products (fish products, milk and milk products, meat and meat products), either by inoculating some liquid mediums (saline alkaline peptonate water – SAPW, saline glucose broth– SGB, phosphate - sorbitol – bile broth after the formula proposed by Mehlman and Aulisio, irgasan ticarcillin potassium chlorate broth - BITP after the formula proposed by De Zuter and col.) or solid mediums (Salmonella-Shigella agar (SS), Hektoen Enteric agar, as well as some mediums with lactose, such as Mac Conkey agar or Drigalski agar) (8, 11).

The tolerance towards the temperature has been tested for three categories (low temperatures, medium temperatures, high temperatures), within each category different levels of temperatures being applied: low temperatures (refrigeration at 0 °C and +4 °C; freezing at –4 °C, -12 °C, -18 °C), medium temperatures (10 °C, 25 °C, 30 °C, 35 °C, 40 °C) and high temperatures (50 °C, 60 °C, 70 °C, 80 °C, 90 °C). The temperatures have been applied on different time periods and substantive scientific data have been obtained for an objective statistic analysis.

Rezultates and Discutions

The behaviors of *Yersinia enterocolitica* strains towards different temperatures, depending on certain time periods, is synthetic presented in charts no. 1, 2, 3 and 4.

Table 1. The Yersinia enterocolitica strain tolerance at refrigeration and freezing temperatures

Sample	-18°C		-12°C		-4°C			0°C		+4°C	
	10 days	20 days	10 days	20 days	5 days	10 days	15 days	2 days	4 days	2 days	4 days
5 SAPW samples with 10^6 cells/ml strain 2732c ($Ag\ O_3$)	10^2 10^1 10^4 10^3 10^1	10^2 10^1 10^3 10^2 10^1	10^4 10^5 10^5 10^4 10^5	10^2 10^3 10^4 10^3 10^3	10^4 10^5 10^5 10^4 10^2	10^4 10^5 10^5 10^4 10^2	10^2 10^1 10^4 10^3 10^1	10^5 10^5 10^6 10^4 10^5	10^4 10^5 10^3 10^4 10^3	10^6 10^6 10^6 10^6 10^7	10^7 10^6 10^6 10^7 10^8
5 SGB samples with 10^6 cells/ml strain 1765pl ($Ag\ O_9$)	10^2 10^1 10^4 10^1 10^3	10^2 10^1 10^2 10^1 10^3	10^4 10^3 10^5 10^4 10^5	10^2 10^1 10^4 10^1 10^3	10^4 10^3 10^5 10^4 10^5	10^4 10^3 10^5 10^4 10^5	10^2 10^1 10^4 10^1 10^3	10^4 10^5 10^5 10^6 10^5	10^4 10^3 10^5 10^4 10^5	10^4 10^3 10^5 10^4 10^5	10^7 10^6 10^5 10^7 10^8

5 BITP samples with 10^6 cells/ml strain 1946p (Ag O_2)	10^3	10^3	10^5	10^3	10^5	10^5	10^3	10^5	10^5	10^7	10^8
	10^4	10^4	10^5	10^4	10^5	10^5	10^4	10^6	10^5	10^6	10^9
	10^2	10^1	10^6	10^2	10^6	10^6	10^2	10^6	10^6	10^5	10^9
	10^2	10^2	10^4	10^2	10^4	10^4	10^2	10^5	10^5	10^7	10^7
	10^3	10^2	10^6	10^3	10^6	10^6	10^3	10^6	10^5	10^6	10^{10}

The comparative analysis of the obtained results allowed the achievement of some very useful statistical data, with substantive practical application especially in the food industry field. The *Y. enterocolitica* O_9 strain had a similar response with the strains of O_3 serotype for the same food product. The study of *Y. enterocolitica* strains resistance to low temperatures (refrigeration and freezing), proves that these bacteria are not sensitive to temperatures below 0^oC, but significantly decrease at temperatures below 0 ^oC, while at refrigeration temperatures they can even multiply with 2 or 3 logarithms.

Table 2. The Yersinia enterocolitica strains tolerance in food products at freezing and refrigeration temperatures

The sample experimentally contaminated	**-18^{o}C**		**-12^{o}C**		**-4^{o}C**			**0^{o}C**		**+4^{o}C**	
	10 days	20 days	10 days	20 days	5 days	10 days	15 days	2 days	4 days	2 days	4 days
5 fish samples with 10^6 cells/ml strain 1025pa (Ag O_2)	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^5	10^4	10^6	10^7
	10^4	10^3	10^3	10^1	10^5	10^5	10^4	10^6	10^5	10^6	10^6
	10^3	10^2	10^3	10^2	10^4	10^4	10^3	10^6	10^6	10^6	10^8
	10^3	10^2	10^2	10^2	10^5	10^5	10^4	10^6	10^5	10^6	10^7
	10^1	10^1	10^1	10^1	10^2	10^2	10^1	10^1	10^1	10^6	10^8
5 pig meat samples with 10^6 cells/ml strain 2732c (Ag O_3)	10^1	10^1	10^1	10^1	10^2	10^2	10^1	10^5	10^5	10^6	10^6
	10^2	10^2	10^2	10^2	10^5	10^5	10^2	10^5	10^4	10^6	10^6
	10^4	10^1	10^3	10^1	10^5	10^5	10^4	10^4	10^3	10^6	10^7
	10^3	10^2	10^2	10^1	10^4	10^4	10^3	10^6	10^5	10^6	10^7
	10^2	10^2	10^2	10^2	10^3	10^3	10^2	10^5	10^5	10^6	10^6
5 bovine meat samples with 10^6 cells/ml strain 816b (Ag O_3)	10^4	10^2	10^4	10^2	10^5	10^5	10^4	10^6	10^5	10^6	10^7
	10^3	10^1	10^3	10^3	10^4	10^4	10^3	10^5	10^5	10^6	10^7
	10^4	10^2	10^2	10^1	10^5	10^5	10^4	10^5	10^5	10^6	10^8
	10^4	10^1	10^4	10^3	10^5	10^5	10^4	10^4	10^4	10^6	10^9
	10^2	10^1	10^2	10^1	10^4	10^4	10^2	10^6	10^6	10^6	10^8
5 poultry meat samples with 10^6 cells/ml strain 1946p (Ag O_2)	10^2	10^1	10^2	10^2	10^3	10^3	10^2	10^5	10^5	10^6	10^6
	10^4	10^2	10^2	10^1	10^5	10^5	10^4	10^5	10^6	10^6	10^8
	10^3	10^1	10^1	10^1	10^4	10^4	10^3	10^4	10^5	10^6	10^6
	10^3	10^1	10^3	10^1	10^5	10^5	10^3	10^6	10^3	10^6	10^7
	10^2	10^2	10^2	10^2	10^4	10^4	10^2	10^6	10^4	10^6	10^8
5 milk samples with 10^6 cells/ml strain 1765pl (Ag O_9)	10^1	10^1	10^1	10^1	10^3	10^3	10^1	10^6	10^5	10^6	10^7
	10^2	10^2	10^2	10^2	10^4	10^4	10^2	10^6	10^6	10^6	10^6
	10^1	10^1	10^1	10^1	10^4	10^4	10^1	10^5	10^6	10^6	10^7
	10^1	10^1	10^1	10^1	10^3	10^3	10^1	10^6	10^5	10^6	10^8
	10^2	10^2	10^2	10^2	10^2	10^2	10^1	10^6	10^6	10^6	10^9

Y. enterocolitica O_3 is sensitive to freezing more in solid food products compared to liquid food products. Thus, *Y. enterocolitica* Ag K 2 was encountered in milk frozen at $–18^0C$ at values of 10^6cells/ml after 20 days, and 10^5cells/ml after 30 days. This observation comes

to support Schiemann (13), who demonstrates that the number of *Y. enterocolitica* cells is not significantly reduced in milk frozen at -20^0C, for 30 days. For solid products frozen at -18^0C, the number of Y. enterocolitica cells is reduced with 2 logarithms in bovine meat and only with one logarithm in pig meat after 20 and 30 days. In poultry, at -18^0C, after 20 and 30 days, the bacteria population density reduction was low on the surface of the poultry meat samples analyzed (the reduction was of 2 logarithms). The number of *Y. enterocolitica* cells was maintained constant or decreased with 1-2 logarithms at -12^0C and -4^0C, both in milk samples as well as in meat samples that were analyzed. *Y. enterocolitica* multiplied at refrigeration temperatures (0^0C and 2-4^0C); the multiplication was more rapid on raw bovine meat (2 logarithms) compared to the raw pig meat and raw poultry meat, raw milk respectively, products for which it was noticed the rise of the cells number with maximum 1 logarithm, or even standing.

Table 3. Yersinia enterocolitica strains growing at temperatures between 10°C and 40°C

Strain	Medium	At 10°C	At 25°C	At 30°C	At 35°C	At 40°C
2732c strain ($Ag O_3$)	**SAPW**	+	+	+	+	+
	SGB	+	+	+	+	+
	BITP	+	+	+	+	+
1765pl strain ($Ag O_9$)	**SAPW**	+	+	+	+	+
	SGB	+	+	+	+	+
	BITP	+	+	+	+	+
1946p strain ($Ag O_2$)	**SAPW**	+	+	+	+	+
	SGB	+	+	+	+	+
	BITP	+	+	+	+	+

The studies regarding the tolerance to medium temperatures conclude on the ability of most of *Yersinia enterocolitica* strains of growing at temperatures between 10°C and 40°C. This proves *Yersinia's* ability of occupying ecological niches in all types of environments and biological products and the ability of multiplication in conditions that are not favorable for other organisms.

Table 4. The Yersinia enterocolitica strains tolerance at high temperatures

Sample / time	At 50°C		At 60°C		At 70°C		At 80°C		At 90°C	
	5 min.	10 min.	5 min.	10 min.	5 min.	10 min.	5 min.	10 min.	5 min.	10 min.
strain 1025pa 10^9cells/ml	10^9	10^6	10^4	10^2	10^2	10^1	10^1	0	0	0
strain 2732c 10^9cells/ml	10^9	10^6	10^3	10^1	0	0	0	0	0	0
strain 816b 10^9cells/ml	10^9	10^6	10^2	10^1	10^1	0	0	0	0	0
strain 1946p 10^9cells/ml	10^9	10^7	10^4	10^1	0	0	0	0	0	0

strain 1765pl 10^9cells/ml	10^8	10^6	10^4	10^2	10^1	0	0	0	0	0
strain 1432c 10^9cells/ml	10^9	10^7	10^6	10^2	10^2	0	10^1	0	0	0
strain 2696b 10^9cells/ml	10^9	10^6	10^2	10^1	10^1	0	0	0	0	0
strain 2682p 10^9cells/ml	10^9	10^7	10^3	10^1	0	0	0	0	0	0
strain 3788pl 10^9cells/ml	10^8	10^4	10^2	10^1	0	0	0	0	0	0
strain 3781pl 10^9cells/ml	10^8	10^6	10^2	0	0	0	0	0	0	0

The resistance at high temperatures is very low, practically at 70 ^oC all the strains being destroyed in maximum 10 minutes. These results are in line with those obtained by other research teams that have studied a similar theme (9, 10, 15).

Conclusions

3.1. Freezing temperatures (–18 ^oC, - 12 ^oC) applied for maximum 20 days do not destroy *Yersinia enterocolitica* species regardless the substratum/medium.
3.2. Refrigeration temperatures (0 – 4 ^oC) do not destroy *Yersinia* strains, ensuring even a significant rise of bacteria number / ml or g substratum/medium, in certain cases with 2-3 logarithms.
3.3 Medium temperatures (between 10 – 40^oC) ensure optimum multiplication for most of the *Yersinia enterocolitica* strains tested, proving also their ability of direct multiplication in food products stored at inappropriate temperatures or in raw materials which are not rapidly introduced in low temperature environments.
3.4. High temperatures, above 50 – 60^oC, ensure the rapid destroying of *Yersinia* strains, practically all the strains being destroyed in maximum 10 minutes at a temperature of above 70 ^oC.

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