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HEAT RESISTANCE OF *Pseudomonas aeruginosa* IN PREPARATIONS AT THE BASE OF CUCUMBER, TOMATO AND LETTUCE AS AFFECTED BY PH AND SODIUM CHLORIDE

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Abstract

The effect of the acid and the osmotic stress on the heat resistance of *Pseudomonas aeruginosa* MC₁ was studied at 63 °C in preparations at the base of cucumber, tomato and lettuce adjusted to various pHs (2, 3 and 4) or various NaCl concentrations (2, 4 and 6%). In the second study, the combined effect of pH and NaCl on the thermal inactivation of *P. aeruginosa* cells was determined. The heat resistance of *P. aeruginosa* cells was more rapid at pH 2 after 10 min of thermal shock for the three preparations. On the contrary, the heat resistance increased with increasing the pH values. At pH 3 or 4, viable cells were detected after 30 min of treatment when the cells inoculated in preparation of tomato. Addition of NaCl, was involved in the protection of cells against heat inactivation. Indeed, the best heat resistance of *P. aeruginosa* MC₁ strain was obtained in tomato preparation supplemented with 4 or 6% of NaCl. This study indicates that heat resistance of *Pseudomonas aeruginosa* MC₁ strain was lower at pH 2 and 2, 4 or 6% of sodium chloride. In addition, no viable cells were recovered after 1 min of treatment in lettuce preparation.

Keywords: *Pseudomonas aeruginosa*, pH, salt concentration, heat resistance.

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Introduction

Pseudomonas aeruginosa is part of the Psychrotrophic flora, they responsible for alterations of the commercial quality of food-stuff. The bacterium represents one of the three greater causes of opportunist infections at the human one (Salyers & Whitt, 2002). It is very pathogenic for the weakened subjects (Briand, 1991; Rahme et al., 2000). *Pseudomonas aeruginosa* is able to survive in a great diversity of food products of animal or vegetable origin (Rahme et al., 2000).

Exposure to heat has long been recognized as a primary method for preserving foods, typically resulting in the production of either a pasteurized or a commercially sterile food product, or the more classic cooking of food in preparation for consumption (Cassens, 1994). But then, thermal inactivation of bacteria is affected by numerous

intrinsic factors. In general, resistance is higher in meat products than in buffer solutions, peptone-agar, or other model media (Bell & DeLacy, 1984; Blankenship & Craven, 1982; Ghazala et al., 1995; Juneja et al., 1995a; Murphy et al., 1999). In any medium, there is typically an optimal pH for maximum bacterial heat resistance (Chiruta et al., 1997; Gaillard et al., 1998; Juneja et al., 1995b; Juneja & Eblen, 1999).

Water activity (and/or moisture content) of the product is widely known to be a controlling factor in microbial growth; however, its effect on thermal inactivation is less obvious, but extremely important. Other study reported that *Salmonella* can survivors on the surfaces of fully-cooked, dry roasted beef and suggested that thermal resistance was enhanced by the reduction in water activity (a_w) near

the meat surface. However, very few studies have quantified the effects of moisture content or a_w on microbial inactivation. Kirby and Davies (1990) dehydrated cultures of *S. Typhimurium* and reported an increased thermal resistance; however, they were heating pure cultures rather than a food product.

The study reported here was undertaken to investigate the thermal inactivation of *P. aeruginosa* cells inoculated into preparations at the base of cucumber, tomato and lettuce adjusted to various pH and salt concentrations.

Materials and Methods

Bacterial strains and growth conditions

The *P. aeruginosa* MC₁, used in this study was isolated from the ground water of M'nasra Kénitra (Morocco), by direct plating onto cetrimide agar medium. Identification was confirmed by Gram strain morphology, a positive oxidase reaction and by using the API 20E diagnostic system. Strain was stored frozen (-20 °C) in tryptic soy broth-glycerol 50% (vol/vol). Each stock culture of *P. aeruginosa* MC₁ was cultured twice in tryptic soy broth overnight at 30°C before used in the present experiments.

Inoculum preparation

The inoculum was prepared by pipetting 1 ml of an overnight static culture (30°C) of *P. aeruginosa* MC₁ into 9 ml of tryptic soy broth. This preparation was incubated at 30°C with shaking for 18 h. They were then harvested by centrifugation (3 min at 13,000 x g) and washed in an equal volume of sterile saline water (0.9% [wt/vol] NaCl in distilled water). After a second washing, one millilitre of washed cell suspension (approximately 10⁸ cells. ml⁻¹) was used to inoculate 99 ml of medium.

Preparation of survival media

The thermal inactivation of *P. aeruginosa* MC₁ was carried out in preparations at base of cucumber, tomato and lettuce. 100 g of cucumber, tomato and lettuce rinsed beforehand with sterile distilled water, are cut and homogenized with 400 ml of water distilled using a Moulynex Robot. After preparations of the mediums, they are sterilized at 110 °C for 10 min.

Effect of sodium chloride or pH on the heat resistance of P. aeruginosa

The heat resistance of *P. aeruginosa* was evaluated in preparations at base of cucumber, tomato and lettuce adjusted to various pHs or to different NaCl concentrations.

- **pH.** Sterile Erlenmeyer flasks (250 ml) containing the 99ml of test media (cucumber, tomato and lettuce) adjusted to various pHs (2, 3 or 4) with HCl 1N, were prepared and stored at the room temperature until inoculation the following day. Thus, one ml of 18 hours cultures of *P. aeruginosa* strain MC₁ was added to the test media to give an initial level of approximately 10⁸ CFU/ml. Following inoculation, all treatments and control were marketed at 63 °C in an aquatherm water bath shaker. After heating during 1, 10, 20 or 30 min, tempered flasks were cooled in an ice-water mixture.

- **NaCl.** Erlenmeyer flasks (250 ml) containing the 99ml of test media (cucumber, tomato and lettuce) adjusted to different NaCl concentrations (2, 4 or 6% [wt/vol]), were inoculated with 1 ml of 18 hours cultures of *P. aeruginosa* strain MC₁ (initial cell density was 10⁸ CFU ml⁻¹), then they incubated at 63 °C in an aquatherm water bath shaker. After heating during 1, 10, 20 or 30 min, tempered flasks were cooled in an ice-water mixture.

Effect of combination of pH and NaCl on the heat resistance of P. aeruginosa

This experiment was carried out to analyze combined effect of pH and NaCl on the thermal inactivation of *P. aeruginosa* in preparations at the base of cucumber, tomato and lettuce. Erlenmeyer flasks (250 ml) containing 99 ml of test media adjusted to various pHs (2, 3 or 4) and different NaCl concentrations (2, 4 or 6%) were inoculated with 1 ml of 18 hours cultures of strain MC₁ (initial cell density was 10⁸ CFU ml⁻¹), then they tempered at 63 °C in an aquatherm water bath shaker. After heating during 1, 10, 20 or 30 min, the tempered flasks were cooled in an ice-water mixture. Untreated sample was used as a control.

Viable counts

Cell suspensions were serially diluted in saline water (0.9% [wt/vol] NaCl in distilled water) and plated onto tryptic soy agar. Colonies were counted after the plates were incubated at 30°C for 48 h. The survival curves were based on mean values obtained from two experiments.

Results

Effect of pH

The effect of pH on the heat resistance of *P. aeruginosa* MC₁ is shown in Fig. 1. Heat resistance was assessed by comparison of survival levels after exposure of cells at 63 °C in preparations at base of cucumber, tomato and lettuce acidified to pH 2, 3 or 4 by using cells taken from the stationary phase of growth. The heat resistance of *P. aeruginosa* MC₁ was low at pH 2 than at pH 3 or 4. At pH 2 the viability of the cells decreased rapidly after 10 min of thermal shock with a reduction of 4.10, 6.28 and 6.70 units log₁₀

respectively in lettuce, cucumber and tomato. After 20 min of treatment the viability of cells was completely inhibited in cucumber. However, when the cells are cultivated in tomato or lettuce the time needed to decline the numbers of survivors to undetectable number was 30min.

At pH 3 or 4 the number of viable *P. aeruginosa* cells decreased by 1 to 4.9 units log₁₀ after 10 min of thermal treatment for the three preparations. When the cells inoculated in cucumber no viable cells were recovered after 20 min of shock. In contrary, viable cells were detected after 30 min of treatment in tomato.

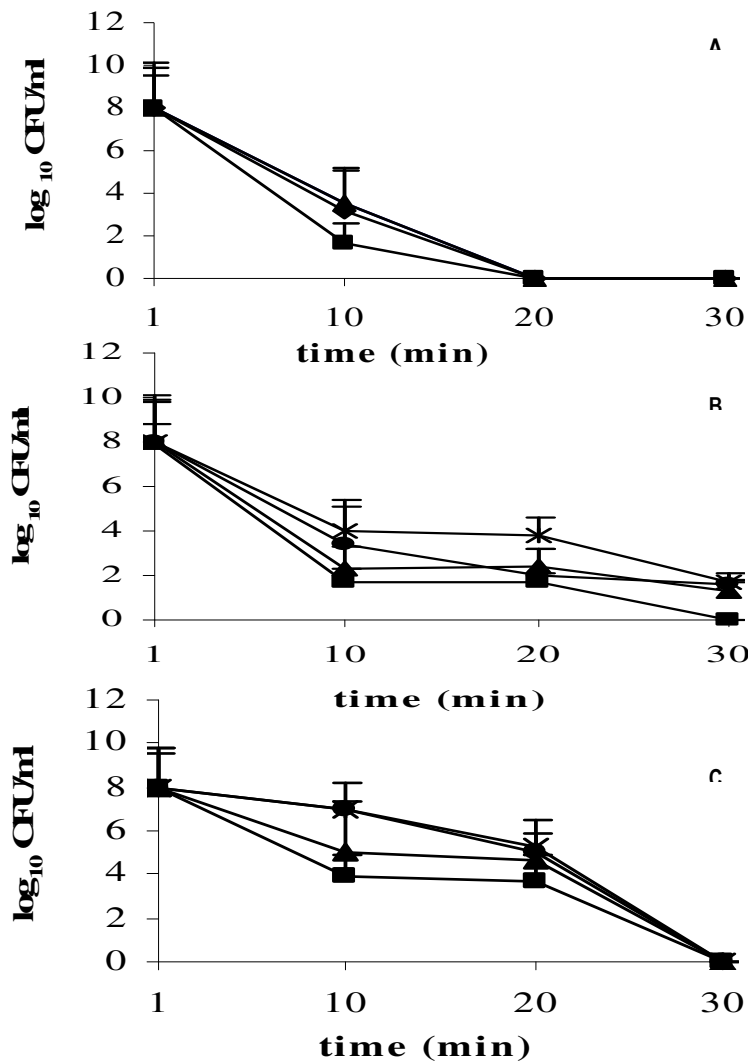


Figure 1. Thermal inactivation of *P. aeruginosa*. cells inoculated into preparations at the base of cucumber (A), tomato (B) and lettuce (C) adjusted to various pH. Control —*, pH=2 —■—, pH=3 —▲—, pH=4 —●— .

Effect of sodium chloride

The effect of sodium chloride on the heat resistance of *P. aeruginosa* MC₁ at 63°C can be seen in Fig 2. Generally presence of sodium chloride was involved in protection of cells against heat inactivation. The presence of NaCl at 2 or 4%, conferred a protective effect on the cells in the preparations at the base of cucumber. When the cells were inoculated in the

preparations at the base of tomato presence of 4 or 6%, sodium chloride contributes to the increase in the viability of the cells: viable cells were recovered after 30 min of treatment. On the contrary, no protective effect was found when the *P. aeruginosa* cells were inoculated in the preparations at the base of lettuce.

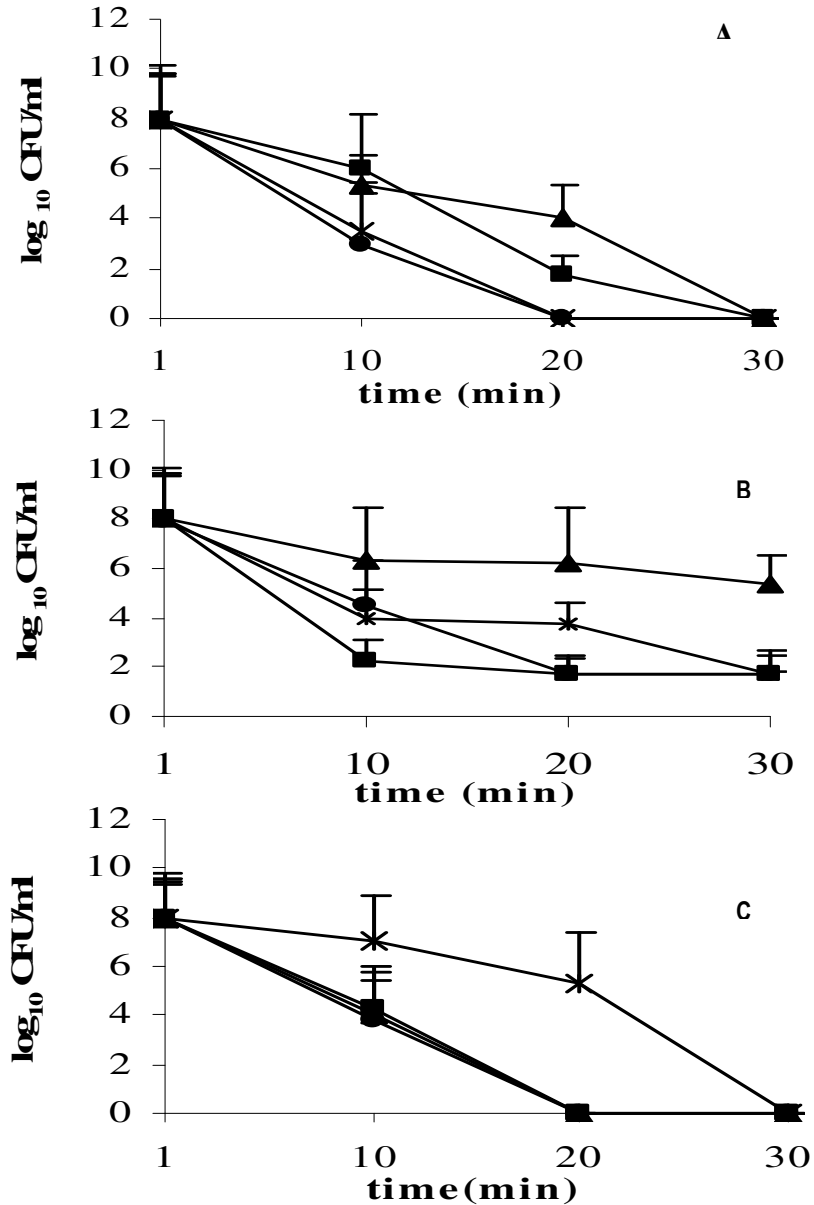


Figure 2. Thermal inactivation of *P. aeruginosa*. cells inoculated into preparations at the base of cucumber (A), tomato (B) and lettuce (C) adjusted to different NaCl concentrations. Control (*), 2% NaCl (■), 4% NaCl (▲), 6% NaCl (●).

Table I

Thermal inactivation of *P. aeruginosa* MC1. Cells were grown to stationary phase in TSB, suspended (10^8 CFU ml⁻¹) in the three preparations at base of tomato, cucumber and lettuce adjusted to all combinations of NaCl (2, 4 or 6%) and pHs (2, 3, or 4), and then heated at a temperature of 63 °C.

pH	% of NaCl	Heating time (min)	Population density (log ₁₀ CFU ml ⁻¹)		
			Cucumber	Tomato	Lettuce
2	2	1	1,7	2,3	0,6
		10	-	-	-
		20	-	-	-
		30	-	-	-
	4	1	1,9	2,18	-
		10	-	1,95	-
		20	-	0	-
		30	-	0	-
	6	1	-	2,08	-
		10	-	1,78	-
		20	-	-	-
		30	-	-	-
3	2	1	1,6	4,6	2
		10	-	4,3	-
		20	-	-	-
		30	-	-	-
	4	1	2,8	4	2,04
		10	-	2,48	-
		20	-	-	-
		30	-	-	-
	6	1	2	4,4	2,84
		10	-	2,95	-
		20	-	1,95	-
		30	-	-	-
4	2	1	6,48	7,04	2,08
		10	-	6,85	-
		20	-	3,11	-
		30	-	1,74	-
	4	1	3,85	7,04	2,78
		10	-	6,85	1,48
		20	-	3,11	-
		30	-	1,74	-
	6	1	2,78	4,04	3,3
		10	-	3,3	1,78
		20	-	-	-
		30	-	-	-

Combined effect of the pH and NaCl on thermal inactivation of P. aeruginosa

The combined effect of the pH (2, 3 or 4) and NaCl (2, 4 or 6 %) on the heat resistance of *P. aeruginosa* MC₁ at 63 °C was assessed by the comparison of survival level after 30 min of heating (Table I). In the three preparations (tomato, cucumber and lettuce), the lethal effect of heat treatment was much enhanced at pH 2 and 2, 4 or 6 % of sodium: the viability of cells was completely inhibited after 1 min of treatment.

At pH 3 a high degree of heat resistance was obtained only in the preparations at base of tomato with 6% of NaCl, viable cells were detected after 20 min. Also survival was demonstrated in the preparations at base of tomato adjusted at pH 4 and 2 or 4 % of sodium chloride after 30 min of treatment. However, 6% of NaCl was necessary to decline the numbers of survivors to undetectable number, after 10 min of shock.

Discussion

This study, showed clearly that low pH, may affect the thermal resistance of *P. aeruginosa*. Inactivation of *P. aeruginosa* MC₁ strain was more rapid at pH 2 after 10 min of thermal shock for the three preparations. However, at pH 3 or 4, viable cells were detected after 30 min of treatment when the cells inoculated in preparation of tomato. Previous studies have shown that bacteria cells were less resistant to heat in lower pH. Oulkheir et al. (2007) reported that the thermal inactivation of two strains of *E. coli* was more rapid at 63 °C in TSB acidified to pH 2.5. However, at pH 4.5 or 6, viability of both strains was almost similar. All the same, most literature on the inactivation of foodborne pathogens is based on studies conducted with liquid media (Smith, 1995), meat slurries (Abdul-Raouf et al., 1993), or external inoculation of meat (Fu et al., 1995; Kim et al., 1994; Manu-Tawiah et al., 1993; Nychas & Tassou, 1996). For example, most of the published inactivation data for *Listeria* were developed with liquid media (Stephens et al., 1994; Membré, et al., 1996). Studies have shown that following heat treatment, many microorganisms show loss of permeability and increased sensitivity to some compounds to which they are normally resistant (Ray, 1996). Sublethal heat stress results in injury of the cell membrane, cell wall, DNA (strand break), ribosomal RNA (degradation), and enzymes (denaturation). Death occurs from damages in some vital functional and structural components, especially if the injury is irreparable (Ray, 1996).

This study has confirmed that the presence of sodium chloride was involved in protection of cells against heat inactivation. Indeed, the best heat resistance of *P. aeruginosa* MC₁ strain was obtained in tomato preparation supplemented with 4 or 6% of NaCl. Our observations are in agreement with those of Oulkheir et al. (2007), who reported that addition of NaCl to tryptic soy broth, afforded a protective effect against inactivation of *E. coli*. Other authors Krotola and Conner (1997) reported that heat resistance of *E. coli* cells inoculated in meat of turkey was significantly higher in presence of 8 % NaCl, 4 % sodium lactate and 0.5% of polyphosphates than of those inoculated without these additives.

The data presented in this work reveal that the lethal effect of heat treatment was much enhanced at pH 2 and 2, 4 or 6% of sodium chloride. In addition, no viable cells were recovered after 1 min of treatment in lettuce preparation. A previous study (Clavero & Beuchat, 1996), had shown that the sodium chloride did not have a protective effect when the *E. coli* cells were heated to 52 °C in TSB adjusted at an $a_w = 0.95$ (ca. 8.5 NaCl) and pH 6,

5.4 or 4.8. Oulkheir and al. (2007) however, confirmed that the sodium chloride have a protective effect when the *E. coli* cells were heated to 63 °C in TSB adjusted at pH 4.5 or 6 and 2, 4 or 8% NaCl.

It has been reported that a connection between the synthesis of heat shock proteins (HSPs) and the development of thermotolerance has also been found (Abee & Wouters, 1999). For example, it has been demonstrated that mild heating triggers the activation and expression of new groups of genes, and the consequent synthesis of HSPs (Knöchel & Gould, 1995). In the presence of these proteins microorganisms can develop greater resistance to subsequent heating at higher temperature (Ray, 1996).

In conclusion, heat inactivation data supplied in this study reveal that the product factors, such as pH has an effect on thermal resistance of *P. aeruginosa*. So, the presence of sodium chloride could be requisite to survival or repair damage associated with heat stress.

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