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Effect of Curing Salts and High Temperature on Freeze-Injured Cells of *Pseudomonas aeruginosa*

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Abstract: Cells of *Pseudomonas aeruginosa* when frozen at -20°C became injured. The study of the effect of high temperature on these freeze injured cells revealed that injured cells were sensitive to high temperature and they needed shorter time to lose their viability than non-injured cells. Also it was found that storage at -20°C of *P. aeruginosa* increased the lag phase time of the growth of this bacterium, and the injured cells became sensitive to sodium chloride and sodium nitrate. These two salts retarded the growth of injured cells. On the other hand sodium nitrite up to 800 ppm had no effect on injured cells.

Key words: Curing salts, Freezing, Injury, Viability

Introduction

It is well known that one of the important methods of meat preservation is low temperature. Low temperature has adverse effects on the bacteria stored at it. It may cause death or injury to these bacteria (Mossel *et al.*, 1980). The importance of presence of injured cells had been reported by other authors (Busta, 1976, Russell, 1991). Injured cells as defined by Clark and Ordal (1969) are those cells, which can form colonies on enriched media, but not on stressing media.

Another method to increase shelf life of processed meat is achieved by using curing salts such as sodium chloride, nitrate, nitrite or nitric oxide (Gracey, 1982). The effect of sodium chloride, sodium nitrate and sodium nitrite on the growth of *Clostridium perfringens* was tested by El Sanousi (1975). In a study for the investigation of the response of lactobacillus curvatus LTH 1174(a strain isolated from fermented sausage and produces a listericidal bacteriocin) to sodium nitrite in terms of cell growth and bacteriocin production. The result showed that this strain was highly sensitive to nitrite even at a concentration of 10 ppm, where it inhibited its growth and both volumetric and specific bacteriocin production. This inhibitory effect of sodium nitrite was at least partially masked under anaerobic conditions (Verluyten *et al.* (2003). The effect of sodium chloride on the growth of 24 lactic acid bacteria strains isolated from vacuum-packed cooked ring sausages was examined by Korkeala *et al.* (1992). They found that the bacterial growth was enhanced by 1-2% (w/v) of added sodium chloride, while concentrations above 3% (w/v) had a clear inhibitory effect. Paludan *et al.* (2002). Studied the effects of different salt concentrations on decrease in pH and on microflora composition during fermentation of Plaa-Som (a Thai fermented fish product). They found that the fermentation of Plaa-Som was delayed by a salt level of 9% due to inhibition of lactic acid bacteria growth. In a study to determine the influence of curing salts (Nacl and $NaNo_2$) on the destruction of *E. coli* 0157:H7 during Lebanon bologna processing, Chikthimmah *et al.* (2001) found that destruction of *E. coli* 01157: H7 during heating of fermented product to 46.1 °C was significantly reduced by presence of 3.5% Nacl and 156 ppm $NaNo_2$ compared to product without curing salts ($P<0.01$). However, scanty information is available concerning the effect of curing salts on injured bacteria. Labbe and Duncan (1970) studied the effect of sodium nitrite in the inhibition of outgrowth of heat injured spores of *Clostridium perfringens* and they found that concentrations of 0.02 and 0.01% sodium nitrite prevented outgrowth of heat sensitive and heat resistant spores respectively.

Materials and Methods

The organism used in this study was *Pseudomonas aeruginosa* isolated from beef meat. *Pseudomonas aeruginosa* was identified according to Barrow and Feltham (1993) and Buchanan and Gibbons (1974).

Effect of high temperature on the survival of freeze-injured cells

The media

a) Non selective medium:

Included nutrient agar (Oxoid).

b) Selective medium:
Pseudomonas selective medium (Oxoid) was used.
Maintenance medium:
Nutrient broth (Oxoid).

Procedure: Freeze injured cells were obtained by distributing 18 hours old nutrient broth culture of *P. aeruginosa* in 15 ml amount in sterile screw-capped bottles, then these bottle were stored at -20°C for 24 hours. The bottles were then thawed at room temperature. The thawed cells immediately transferred to water baths with shakers at temperature of 50, 60 and 70°C. Triplicate Samples were removed at 15, 30, 45 and 60minute intervals and inoculated on selective and non selective media and incubated for 24-48 hours at 37°C before checked for the occurrence of growth. Controlled cells in this study included 18 hours old broth cultures of this organism without freezing storage.

Effect of curing salts
The following salts were used: Sodium chloride (Analar), sodium nitrate and sodium nitrite (Hopkin and Williams).

Procedure: Freeze-injured cells of *P. aeruginosa* were obtained as mentioned above, and then 0.1ml of the thawed cells were inoculated into nutrient broth medium with added salts at different concentrations (see Table 2 and 3). These were incubated at 37°C and growth was measured by turbidity at the following intervals, 1, 2, 3, 4, 5, 6, 7, 24 and 48 hours.

Results and Discussion

Temperatures of 50, 60 and 70°C had an injurious effect on the unfrozen cells of *P. aeruginosa* and these heat injured cells showed inability to grow on the selective media used in this study. (Table 1). Such injury caused by heat was reported by Tomlins *et al.* (1972)
Cells injured by freezing were found to be more sensitive to high temperature than the non injured cells e.g. The viability of injured cells incubated at 60°C was completely lost in 45 minutes while non injured cells showed a degree of resistance at that temperature where they continued to be viable even for one hour of incubation (Table 1). Such findings were not unexpected since that freeze-injured cells (-20°C) were under stress and in this study these stressed cells were further exposed to high temperature. This sensitivity of injured cells to high temperature might have advantage when meat containing such injured cells is subjected to cooking.

Table 1: Survival of injured cells of Pseudomonas aenuginosa at different temperature

Temperature	Time (minutes)	Non injured cells		Injured cells	
		Growth on N. A.	Growth on Pseudo. Selective medium	Growth on N. A.	Growth on Pseudo. Selective medium
50°C	15	+ + + +	+ +	+ +	+
	30	+ + + +	+ +	+	+
	45	+ + +	+	+	+
	60	+ + +	+	+	+
60°C	15	+ + + +	+ +	+	-
	30	+ + +	+ +	+	-
	45	+ + +	+	-	-
	60	+ +	+	-	-
70°C	15	+	-	-	-
	30	-	-	-	-
	45	-	-	-	-
	60	-	-	-	-

- No growth; + poor growth; + + moderate growth; + + + good growth; + + + + confluent growth.
N. A. Nutrient agar.

Growth studies on injured cells of *P. aeruginosa* on liquid media containing salts resulted in marked prolongation of the lag phase and increase in time for reaching the maximum yield e.g. the lag phase time on the medium with no added sodium nitrate was 2 hrs for non injured cells comparing to 6 hrs. for injured cells. This increase in the time of the lag phase of injured cells had been previously reported by Busta (1976). Studies on the effect of sodium chloride and sodium nitrate on injured cells showed that these two salts had an adverse effect on the injured cells. They retarded the growth of injured cells

Table 2: Effect of sodium chloride on the growth of freeze injured cells on *Pseudomonas aeruginosa*

% of Nacl	Time in hours at 37°C																			
	1		2		3		4		5		6		7		24		48			
	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured		
0.5	-	-	+	-	+	-	++	-	++	-	+++	-	+++	+	++++	++++	++++	++++		
3	-	-	-	-	-	-	+	-	+	-	++	-	++	-	++++	++++	++++	++++		
4	-	-	-	-	-	-	-	-	-	-	-	-	+	-	++++	+++	++++	++++		
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++++	++	++++	++++		
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	+++	-		
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

- No growth; + poor growth; + + Moderate growth; + + + Good growth; + + + + Confluent growth

Table 3: Effect of sodium chloride on the growth of freeze injured cells on *Pseudomonas aeruginosa*

% of sodium nitrate	Time in hours at 37°C																			
	1		2		3		4		5		6		7		24		48			
	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured	Non injured	injured		
0	-	-	+	-	+	-	++	-	+++	-	+++	+	++++	++	++++	++++	++++	++++		
1	-	-	+	-	+	-	++	-	+++	-	+++	+	++++	++	++++	++++	++++	++++		
2	-	-	-	-	+	-	++	-	+++	-	+++	-	++++	+	++++	+++	++++	++++		
3	-	-	-	-	+	-	++	-	+++	-	+++	-	++++	-	++++	++++	++++	++++		
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	++++	++		

- No growth; + poor growth; + + Moderate growth; + + + Good growth; + + + + Confluent growth

as compared with non injured cells and also inhibited the growth of injured cells in concentrations lower than that needed for control cells. e.g. on 6% Nacl after 48 hrs of incubation at 37°C. there was good growth of non injured cells, whereas on the same concentration of Nacl for the same period of time there was complete inhibition of growth of injured cells (Table 2).

Interest in the use of nitrite in cured meat has been increased since nitrite may lead to formation of nitrosamine, which is implicated as a precursor of carcinogenic materials (Gray and Randol, 1979). In this study, trials were attempted to decrease the concentration of nitrite by subjecting the cells to freezing prior treatment with nitrite. Nitrite in a concentration up to 800 ppm was of no detectable effect on both normal and freeze injured cells (Results are not shown). The reduced activity of nitrite in this study could be due to the influence of the high pH of the culture media used (7.6), since it is well confirmed that the effect of nitrite is mainly pronounced at low pH value (Bushway *et al.*, 1982).

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