RESISTANCE OF PSEUDOMONAS AND BROCHOTHRIX SPECIES TO HIGH HYDROSTATIC PRESSURE

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Background

The novel food preservation technique of high hydrostatic pressure has had renewed interest recently as it increases the shelf life of foods, while leaving the organoleptic properties largely unaltered. Other preservation techniques such as thermal treatments change the organoleptic status of a foodstuff and addition of chemical preservatives are now less welcomed by the consumer.
Pseudomonas spp. and *Brochothrix* spp. are bacteria associated with the spoilage of refrigerated beef and pork (Borch *et al*, 1996). Meat stored at chill temperatures, under aerobic conditions, will have a microflora largely composed of Gram-negative aerobes, with *Pseudomonas* being the dominant genus (Gustavsson and Borch, 1993). *Brochothrix*, a Gram-positive, facultative anaerobe, will be more prevalent under anaerobic conditions, for example in vacuum packed bacon or in comminuted meat products, where it causes souring.

Objective

To investigate the resistance of twenty five strains of *Brochothrix* spp. and fifteen strains of *Pseudomonas* spp. to high hydrostatic pressure in a model system and to prepare a selective medium containing sodium chloride to determine the level of non-injured cells present after pressure treatment. This will enable optimal treatment strategies to be devised, by understanding how the lethality of high hydrostatic pressure differs between strains of the same species. Models will need to be based on the most resistant strains to ensure effective reduction in cell numbers. The use of these two microorganisms will give indications for required high pressure treatments for meat and meat products being stored under different atmospheric conditions.

Methods

Organisms

Isolates were collected from within the Food Science Division, Newforge Lane, two reference strains were obtained, while further wild types were isolated from meat products obtained from a local retail outlet. Isolates were maintained on TSAYE slopes at 5°C and labelled Ps 1-Ps 15 for the fifteen *Pseudomonas* and Bt 1-Bt 25 for the twenty five *Brochothrix*.

Preparation of working culture

A loopful of culture was taken from a TSAYE slope and used to inoculate 10 ml of broth (TSBYE). This was incubated overnight at 25°C. A 10^{-4} dilution of overnight culture was prepared and 1ml of this used to inoculate 100 ml TSBYE. This was incubated for 24h at 25°C.

Determination of sodium chloride level required for injury studies

The inoculum level was determined by preparing a serial dilution and carrying out spread plate counts on TSAYE. Spread plate counts were carried out at appropriate dilutions onto TSAYE + 1, 2, 3, 4 and 5% (w/v) NaCl for *Pseudomonas* (Ps. 1, Ps. 5 and Ps. 10) and TSAYE + 1, 2, 3, 4, 5, 6, 7, 8 and 9% (w/v) NaCl for *Brochothrix* (Bt. 1, Bt 2 and Bt. 23). Plates were incubated at 25°C for 24 h and counts were determined using a plate counter.

Pressure sensitivity of psychrotrophs.

The inoculum level was determined by preparing a serial dilution and carrying out plate counts using the spiral plater, onto TSAYE. Culture (3ml) was triple packed in labelled polyethylene pouches. The samples were treated at 250 MPa for 0, 3, 6, 9, 12, 15, 18, and 21 minutes at 25°C. The contents of the pouch were transferred into a sterile plastic bijou bottle, using a sterile plastic pastette. A number of serial dilutions were prepared and the spiral plater was used to determine bacterial numbers on TSAYE and TSAYE+NaCl. These plates were incubated for 48h at 25°C. Plate counts were carried out using an autoplate counter.

Results and discussion

The optimum NaCl concentration required for a selective medium was determined as 3%(w/v) and 5%(w/v) for *Pseudomonas* and *Brochothrix* respectively. The total counts on the plates were not affected by an increase in salt concentration over the range studied; however, the size of the colonies were affected, with colony size decreasing with increasing salt concentration. Required salt concentration was selected on the %NaCl (w/v) that gave a significant reduction in colony size. *Brochothrix* was more salt tolerant than *Pseudomonas*, this was expected since *Brochothrix* is present in spoiled cured meat products (Borch *et al*, 1996).
The inactivation data for *Pseudomonas* and *Brochothrix* are depicted graphically on Figure 1. Patterson and Kilpatrick, 1998 noted that Gram-positive bacteria tend to be more pressure resistant than Gram-negative bacteria and the results of this study are in agreement with this. *Brochothrix* were more resistant to high pressure under these conditions, than *Pseudomonas*. This could have consequences for vacuum packed meat products where shelf life will be extended, but dominating flora are more pressure resistant. High pressure causes a large degree of sublethal injury, as can be seen in the difference between TSAYE and TSAYE+NaCl counts, therefore surviving cells may be suppressed by other techniques such as low temperature or low A_w, i.e. using the hurdle concept.
The variation of pressure resistance between strains (Table 1.) can be difficult to determine. Depending on total Log_{10} inactivation or rate of inactivation, values differ, especially with *Brochothrix*. Further investigations at higher pressures may aid in distinguishing between resistant and sensitive strains of this Gram-positive microorganism. Patterson *et al*. 1995 also investigated variation in pressure sensitivity between strains and found there to be significant differences within *Escherichia coli* and *Listeria monocytogenes* strains. Significant variation within the strains are, however, indicted by both the b and r values independently.

Conclusion

Brochothrix spp. were more resistant to high pressure and salt than *Pseudomonas* spp. There was significant variability in pressure resistance between strains of both *Pseudomonas* spp. and *Brochothrix* spp. Sublethal injury occured in *Pseudomonas* spp. and *Brochothrix* spp at 250 MPa at 25°C at up to 21 minutes in TSBYE (spent). Further studies on food stuffs will allow validation of this model system and enable treatment regimes to be devised for the food industry.

Pertinent literature

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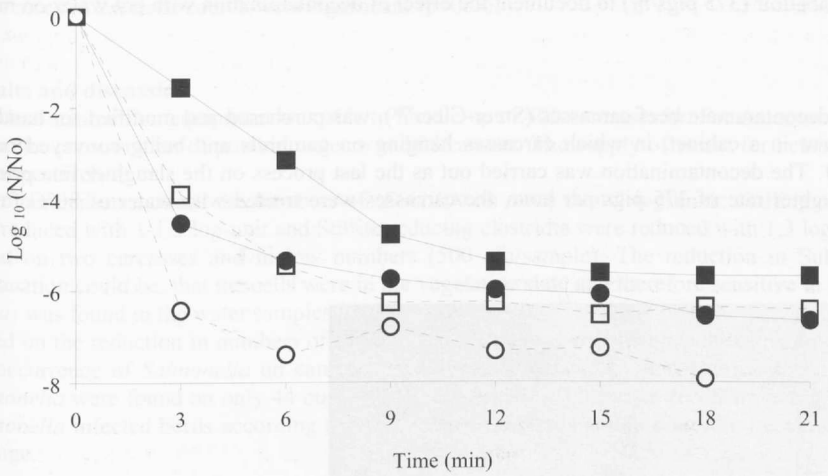

Figure 1. Pressure inactivation of *Pseudomonas* spp. (●-TSAYE, ○-TSAYE+NaCl) and *Brochothrix* spp. (■-TSAYE, □-TSAYE+NaCl) [250MPa at 25°C in TSBYE (spent)].

Brochothrix spp.				*Pseudomonas* spp.			
Strain no.	b	Strain no.	r	Strain no.	b	Strain no.	r
21^{f}	-5.58	2^{h}	0.9456	1^{d}	-5.42	1^{d}	0.846
12^{f}	-5.66	5^{gh}	0.9424	13^{d}	-5.48	11^{cd}	0.767
17^{ef}	-6.01	24^{gh}	0.9329	14^{cd}	-5.72	9^{bcd}	0.753
4^{ef}	-6.02	25^{gh}	0.9313	9^{cd}	-5.78	4^{bcd}	0.725
9^{ef}	-6.18	22^{fgh}	0.9227	11^{cd}	-5.85	6^{abc}	0.687
13^{ef}	-6.24	7^{efgh}	0.9114	10^{bcd}	-6.27	2^{abc}	0.681
23^{ef}	-6.48	8^{efgh}	0.9100	12^{bcd}	-6.28	3^{abc}	0.671
11^{ef}	-6.53	3^{defgh}	0.8990	7^{bcd}	-6.34	10^{abc}	0.670
16^{def}	-6.55	6^{cdefgh}	0.8905	3^{bcd}	-6.39	12^{abc}	0.660
18^{def}	-6.56	19^{cdefgh}	0.8879	4^{bcd}	-6.40	14^{abc}	0.645
10^{def}	-6.57	11^{bcdefg}	0.8810	15^{abc}	-6.80	13^{ab}	0.621
20^{cdef}	-6.60	15^{bcdefg}	0.8786	2^{ab}	-7.06	5^{a}	0.605
14^{cdef}	-6.73	18^{bcdef}	0.8637	8^{ab}	-7.08	15^{a}	0.589
1^{cdef}	-6.79	1^{abcde}	0.8560	6^{ab}	-7.27	8^{a}	0.577
15^{cdef}	-6.81	14^{abcde}	0.8560	5^{a}	-7.70	7^{a}	0.561
7^{cdef}	-6.96	13^{abcde}	0.8534				
3^{cdef}	-7.11	16^{abcde}	0.8496				
6^{bcde}	-7.24	17^{abcd}	0.8431				
19^{bcde}	-7.33	10^{abcd}	0.8412				
8^{bcde}	-7.46	20^{abcd}	0.8357				
25^{bcde}	-7.54	9^{abc}	0.8325				
22^{abcd}	-8.11	4^{abc}	0.825				
24^{abc}	-8.14	12^{ab}	0.8212				
2^{ab}	-8.83	23^{a}	0.8143				
5^{a}	-9.19	21^{a}	0.7946				

Table 1. Comparison of pressure sensitivity of bacterial strains. Inactivation curve $y=b(1-r^{x})$, where b=total Log_{10} decrease and r =rate of inactivation.
(values with similar superscripts are not significantly different)