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# Survival of *Pseudomonas fluorescens* on beef carcass surfaces in a commercial abattoir

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ABSTRACT

The influence of a commercial chilling process (18 h at 10 °C followed by up to 78 h at 2 °C) on *Pseudomonas fluorescens* inoculated on beef carcass surfaces at four sites, neck (NE), outside round (OR), brisket (BR) and foreshank/brisket (FB) before chilling ("hot inoculated") or after chilling for 24 h ("cold inoculated") was investigated. *Pseudomonas* counts increased significantly at all sites on "hot inoculated" carcasses during storage, but on "cold inoculated" carcasses, counts declined or remained unchanged. On hot and cold inoculated carcasses, differences in *Pseudomonas* growth or survival were demonstrated between sites. No clear relationships were observed between *Pseudomonas* growth or survival and chiller relative humidity (RH) or surface water activity ($a_w$) at the different sites. These results were unexpected, and are discussed in relation to environmental factors that affect the growth/survival of *P. fluorescens* on carcass surfaces during chilling i.e. temperature, RH, and the relationship of these parameters to surface water activity ($a_w$).



## 1. Introduction

In the production of tender beef, Irish commercial abattoirs frequently employ biphasic carcass chilling regimes, with initial chilling at a higher temperature, followed by further chilling over a longer period at a lower chiller temperature, e.g. initial chilling at 10 °C for 10–18 h, followed by chilling at 2 °C for a further 24–48 h (Maher et al., 2004; Troy, 1999; White, O'Sullivan, Troy & O'Neill, 2006). The influence of this type of biphasic chilling on bacterial growth on carcasses has not been established, although such regimes will mean that some regions of the carcass surface may remain at relatively higher temperatures (i.e. around 10 °C) for different periods of time at different points on each cooling carcass. Gill, Harrison, and Phillips (1991) and Gill and Bryant (1997) reported the aitch bone pocket as the slowest cooling site on carcasses, with the rump being the next slowest cooling site, within an overall pattern in which larger/thicker parts of the carcass cooled more slowly.

In more general terms, the rates and nature of bacterial growth and associated meat spoilage at different sites on carcasses are influenced by biological factors, such as the composition/size of the mixed bacterial populations present. The nature and organisation of the meat surface and environmental factors including temperature, and surface relative humidity and air flow are also important (Lambert, Smith, & Dodds, 1991; Lebert, Baucour, Lebert, & Daudin, 2005). Surface relative humidity and air flow significantly modulate meat surface water activity ($a_w$) by influencing rates of evaporative water losses and surface drying (Anonymous., 2007; Daudin, 1986; Lebert et al., 2005). Relative humidity (RH) dictates surface evaporation rates during carcass chilling, which in turn controls surface water activity (Anonymous., 2007; Daudin, 1986). Surface $a_w$ may have very significant effects on bacterial growth. For example, the growth of Gram negative bacteria is inhibited below $a_w$ 0.95–0.96 (Anonymous., 2007; Rosset, 1982). Much lower $a_w$ values occur during commercial meat chilling. Thus, Daudin (1986) noted that at low RH (83–88%) and low air velocity (0.2 m/s) the initial surface $a_w$ (1–4 min) of isolated beef tissue can be as low as 0.6–0.7. Subsequent studies (Daudin & Swain, 1990) established that at low RH (61–70%) and temperatures between 6 and 10 °C, reductions in meat surface $a_w$ were controlled by initial drying rate. In turn the initial drying rate is significantly dictated by the rate of air flow across the carcass surface, rather than chiller temperature or RH. The faster the air flow, the lower the initial meat surface $a_w$ values (Lovett, Herbert, & Radford, 1978).

Psychrotropic aerobic genera such as *Pseudomonas* are the most significant agents of spoilage of unpacked chilled stored meats. *Pseudomonas fluorescens* accounts for over 60% of the initial *Pseudomonas* numbers, and this rises to 90% of the total bacterial

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population at the point of spoilage (Gustavsson & Borch, 1993; Lambert et al., 1991; Lebert, Begot, & Lebert, 1998). Perhaps because of this preeminence, many studies have investigated the survival and growth of this species. However, most of these have been carried out with beef pieces or in liquid media (Lebert et al., 1998; Lebert et al., 2005; Neumeyer, Ross, & McMeekin, 1997). Much of the available data, therefore, relates to cut pieces of lean or fat tissue rather than intact carcass surfaces, despite the fact that most of the surfaces of beef carcasses are covered by a membrane or fascia and only a limited amount of lean or fat surface tissue in carcasses presents as cut surfaces. This means that both in terms of heat transfer and water movement to and from meat, smaller pieces of meat with cut surfaces may not represent an appropriate model for bacterial survival/growth on much larger intact beef carcasses, during commercial chilling.

A previous investigation was carried out to determine the survival and growth of *Listeria innocua* on hot and cold beef carcass surfaces. Four sites, the neck, outside round, brisket and foreshank/brisket, were inoculated with *L. innocua* (i) immediately after dressing while hot and (ii) when cold after chilling. After inoculation, carcasses were stored at 4 °C for 72 h. The results indicated that *L. innocua* declined at all sites on hot carcasses after 72 h storage and the reductions were significant on the OR and BR sites, while on cold carcasses, significant reductions occurred at all sites (Prendergast, Rowe, & Sheridan, 2007). While the above investigation was undertaken using a Gram positive psychrotropic pathogen, the present study was concerned with obtaining data on the potential growth/survival of a Gram negative psychrotropic spoilage organism, *P. fluorescens,* at the same four sites on the surface of hot and cold beef carcasses during commercial chilling.

## 2. Materials and methods

### 2.1. Inoculum preparation

*Pseudomonas* fluorescens (ATCC 13525) was made resistant to 10 μg ml$^{-1}$ nalidixic acid (Sigma) by the method of Park (1978), and cultured in Brain Heart Infusion Broth (Oxoid, Basingstoke, UK) at 30 °C for 24 h. The resultant stationary phase cells were recovered, washed three times in Oxoid Maximum Recovery Diluent (MRD) using a model 5403 refrigerated centrifuge at 3000*g* for 10 min at 4 °C (Eppendorf, Hamburg, Germany), and resuspended in MRD to form a washed suspension containing approximately 5 log$_{10}$ cfu ml$^{-1}$. Stationary phase cells were used in the present study, since it has been suggested that cells surviving environmental stresses, such as on animal hides or in faeces, are in stationary phase and these are transferred to carcasses during slaughter and dressing (Sheridan & McDowell, 1998).

### 2.2. Identification and inoculation of carcass sample sites

Carcasses, produced commercially from grass fed steers, were selected at random from a day's production, with a mean side weight 213.5 kg. Four inoculation zones were identified on the right sides of three carcasses i.e. at the neck (NE), outside round (OR), brisket (BR), and the area between the foreshank and brisket (FB). Of these, the NE site was the lean cut muscle surface exposed during carcass dressing, while the other three sites (OR, BR, and FB) were covered by fibrous membranes of connective tissue or fascia.

Immediately after carcass dressing, eight (approximately 5 cm$^2$) sample sites were delineated in each of the above carcass zones, using a sterile coring punch smeared with sterile edible ink. Four of each set of sample sites were not inoculated and used as controls. Each of the other four sample sites in each carcass zone were [a] immediately "hot" inoculated with 5 μl of the above washed suspension (containing approx. 3 log$_{10}$ CFU of *P. fluorescens*) after carcass washing and before entering the chiller or [b] tracked through the commercial chilling process for 24 h and "cold" inoculated in the chill with 5 μl of the above washed suspension (Fig 1).

"Hot" and "cold" inoculated carcass sides were processed in parallel though the commercial biphasic chill process, i.e. 18 h at 10 °C followed by 54 h at 2 °C. Three carcasses were inoculated at the above zones during four visits to the abattoir. Inoculated and non inoculated surface samples were aseptically excised at time zero, 24, 48, and 72 h, and pulsified for 30 s in 10 ml volumes of MRD, in 101 × 152 mm sterile stomacher bags (Seward, Norfolk, UK) using a model PUL 100 Pulsifier™ (Filtraflex, Ontario, Canada).

### 2.3. Parallel broth study

The study also investigated the survival/growth of the above washed cell suspension of *P. fluorescens* in MRD. Thus, a 5 μl volume of the above inoculum (containing approx. 3 log$_{10}$ cfu *P. fluorescens*) was inoculated into each of eight 30 ml polypropylene tubes (Sarstedt, Numbrecht, Germany) containing MRD. Four inoculated tubes were placed in plastic bags which were attached to each of the "hot and cold inoculated" carcasses during chilling and sampled in parallel with the carcass surface, i.e. at time 0, 24, 48, and 72 h (as described above).

### 2.4. Enumeration of P. fluorescens

*P. fluorescens numbers* in pulsified (1 ml) inoculated and uninoculated carcass surface samples and in MRD were estimated by direct plating onto Oxoid *Pseudomonas* agar base containing Oxoid CFC selective supplements and 10 μg ml$^{-1}$ of nalidixic acid, with incubation at 30 °C for 48 h. Numbers of sub lethally damaged *P. fluorescens* cells in pulsified or MRD samples were estimated by comparison of the above direct counts with recovery counts. Recovery counts were obtained by preliminary plating of samples onto a resuscitation medium, Oxoid Tryptone Soya agar (TSA), followed by initial incubation for 2 h at 25 °C, subsequent over pouring with *Pseudomonas* CFC agar containing 10 μg ml$^{-1}$ of nalidixic acid and further incubation at 30 °C for 48 h.

### 2.5. Monitoring of environmental parameters

Carcasses were loaded manually into the chills by factory personnel. The environmental parameters i.e. relative humidity (RH) and ambient temperature in the commercial chiller were recorded every 30 min for up to 96 h using a model HMP 45D Vaisala humidity probe (Vaisala Oyj, Helsinki, Finland) and a Type T copper–constantan thermocouple suspended from the hind leg of a carcass in the centre of the chiller. Temperature and RH data were

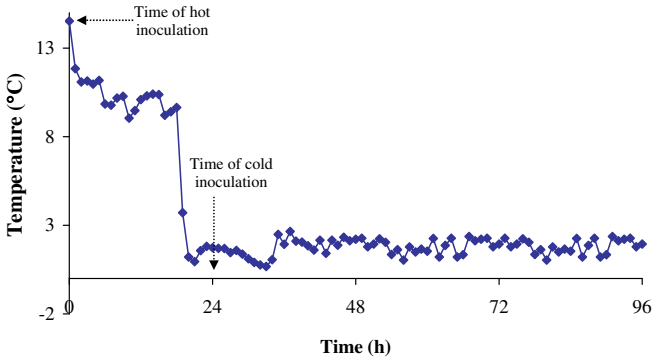


**Fig. 1.** Mean ambient temperature (°C) during carcass chilling.