Journal of Applied Microbiology 2000, **89**, 901–909

Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C

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218/03/00: received 8 March 2000, revised 12 June 2000 and accepted 19 June 2000

E. TSIGARIDA, P. SKANDAMIS AND G-J.E. NYCHAS. 2000. The effect of aerobic, modified atmosphere packaging (MAP; 40% CO_2/30% O_2/30% N_2) and vacuum packaging (VP) on the growth/survival of *Listeria monocytogenes* on sterile and naturally contaminated beef meat fillets was studied in relation to film permeability and oregano essential oil. The dominant micro-organism(s) and the effect of the endogenous flora on the growth/survival of *L. monocytogenes* were dependent on the type of packaging film. The fact that *L. monocytogenes* increased whenever pseudomonads dominated, i.e. aerobic storage and MAP/VP in high-permeability film, and even earlier than on sterile tissue, suggests that this spoilage group enhanced growth of the pathogen. *Brochothrix thermosphacta* constituted the major proportion of the total microflora in MAP/VP within the low-permeability film, where no growth of *L. monocytogenes* was detected either on naturally contaminated or sterile meat fillets. The addition of 0·8% (v/w) oregano essential oil resulted in: (i) an initial reduction of 2–3 $\log_{10}$ of the majority of the bacterial population, with lactic acid bacteria and *L. monocytogenes* indicating the most apparent decrease in all gaseous environments, and (ii) limited growth aerobically and survival/death of *L. monocytogenes* in MAP/VP, regardless of film permeability.

INTRODUCTION

It is well known that the composition (e.g. O_2, N_2 and CO_2) of modified atmosphere systems can be an effective means to restrict and/or inhibit growth of aerobic spoilage organisms of perishable foods such as meat, fish and their products, as well as to sustain the visual quality of red meat (Stanbridge and Davies 1998). The effectiveness of these systems depends, among other factors, on storage temperature and film permeability (McMullen and Stiles 1991; Kotzekidou and Bloukas 1996; Giannuzzi *et al.* 1998). For example, in refrigerated meat, when highly O_2-permeable packaging is used, the spoilage is due to Gram-negative bacteria while, with highly O_2-impermeable packaging, the selection of lactic acid bacteria and *Brochothrix thermosphacta* is evident (Stanbridge and Davies 1998). However, the extended shelf-life of refrigerated products stored under vacuum packaging/modified atmosphere packaging (VP/MAP) conditions has increased concern about the growth/survival of microaerophilic psychrotrophic pathogens (García de Fernando *et al.* 1995). This is particular the case with *Listeria monocytogenes.* However, in the literature there are controversial findings related to the inhibitory effect of VP/MAP systems on the growth of this pathogen in model systems or foods (Hart *et al.* 1991; Marshal *et al.* 1992; Bennik *et al.* 1995; Sheridan *et al.* 1995; Beumer *et al.* 1996; Francis and O'Beirne 1998; Lyver *et al.* 1998). In such packaging conditions, the variability of packaging film properties has not been considered thoroughly for these inconsistencies. Furthermore, listeria, which are widely distributed in meat, poultry, fish and raw agricultural products (García de Fernando *et al.* 1995), have been found to survive even in fermented foods (cheese, yoghurt and sausage) (Zottola and Smith 1991; Uyttendaele *et al.* 1999). Thus, acidification, reduction in

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water activity, addition of preservatives and presence of a competitive microbiota (e.g. lactic acid bacteria) have also been considered as additional tools to prevent the growth of this pathogen in refrigerated foods (Harmayani *et al.* 1993; Jeppesen and Huss 1993; George *et al.* 1996; Fernández *et al.* 1997; Razavilar and Genigeorgis 1998). Since the use of essential oils can be regarded as a 'natural' alternative to chemical preservatives they could be used instead of other 'exo-natural' substances. Indeed, there is considerable interest in the potential use of these compounds as alternative food additives either to prevent the growth of food-borne pathogens or to delay the onset of food spoilage. Many spices, herbs and extracts possess antimicrobial activity that could be attributed to their essential oil fraction (Nychas 1995). The efficacy of these compounds is well established in liquid growth media in comparison to solid foods (Skandamis *et al.* 2000). Only a few studies have proven the successful 'performance' of such natural compounds in foods. This is due to the fact that the addition of essential oil creates a unique ecosystem, which in many cases affects the growth of bacteria (Skandamis *et al.* 2000). The efficacy of essential oils against *L. monocytogenes, Salmonella enteritidis* and *Escherichia coli* has been tested so far in tzatziki, taramasalad, pâté, aubergine salad and fish fillets (Tassou *et al.* 1995, 1996; Koutsoumanis *et al.* 1999; Skandamis and Nychas 2000).

In this study, an effort was made to investigate the effect of oregano essential oil on the population of *L. monocytogenes* as well as on the autochthonous flora of fresh meat fillets stored under VP, MAP or in air at 5 °C using packaging films with different permeabilities. Under these packaging conditions, the behaviour of *L. monocytogenes* was also monitored on sterile meat tissue in order to substantiate any possible effect of the background flora of naturally contaminated meat fillets on the growth/survival of this pathogen (Jay 1996).

MATERIALS AND METHODS

Preparation of beef

For each single experiment, two batches of beef muscle (approximately 5 and 10 kg, respectively) were obtained from a local processing plant and transported under refrigeration to our laboratory within 1 h. The meat of the first batch was cut into 180 pieces (25 g each piece, thickness 0·8 cm). To obtain meat samples without the endogenous microflora the following procedure was performed: the meat surface of the second batch was sprayed with 100% alcohol and burnt with a gas burner in order to reduce the initial microbial load. The burnt surface tissue was removed aseptically and the sterile tissue below was excised and cut into 180 pieces as described above.

Extraction of essential oil

Dried oregano (500 g) (*Origanum vulgare*) was purchased from a local retail spice market, placed in a 2-l flask and 1 l distilled water was added. A continuous steam distillation extraction was performed for approximately 3 h and the oil collected and stored at 4 °C until use.

Preparation of inoculum and inoculation procedure

Listeria monocytogenes strain Scott A was kindly provided by Dr Eddy Smid (ATO-DLO, Bornsesteeg 59, AA-Wageningen, The Netherlands). The stock culture was maintained on Nutrient Agar (1.05450; Merck, Darmstadt, Germany) slopes at 4 °C and subcultured once a week. To obtain the inoculum, the micro-organism was subcultured twice in a 250-ml conical flask containing 100 ml Brain Heart Infusion Broth (1.13825; Merck). The flask was incubated aerobically without agitation at 30 °C for 18 h. Cells were harvested and washed by centrifugation with sterile quarter-strength Ringer's solution (Lab M, Bury, UK). Each meat fillet from both batches was placed in a sterile, 17-cm diameter, plastic Petri dish and dipped in an appropriate volume of bacterial suspension in Ringer's solution yielding approximately 3·5 $\log_{10}$ cfu g^{-1}. In 180 samples (90 with background flora and 90 sterile) appropriate volumes of oregano distillate were added by surface dipping, yielding 0·8% (v/w) essential oil per sample. The selected concentration of oregano essential oil was organoleptically acceptable after cooking of uninoculated raw beef.

Packaging

For both batches (either in the presence or absence of competitive microflora), equal numbers of inoculated samples with or without essential oil were packaged individually under three commercially used packaging conditions, i.e. air, 40% CO_2/30% O_2/30% N_2 (MAP) and vacuum (VP), and stored at 5 °C. For the aerobic storage, the samples were placed in sterile Petri dishes. Two films with different values of O_2 permeability were used: (i) a highly O_2-permeable film with an O_2 transmission rate of 3600 cm^3 m^{-2} 24 h^{-1} at 23 °C and 75% r.h. and (ii) a low O_2-permeable film with an O_2 transmission rate of 4·5 cm^3 m^{-2} 24 h^{-1} at 23 °C and 75% r.h. The meat samples were either modified atmosphere or vacuum packaged using a HencoVac 1700 machine (Howden Food Equipment B.V., The Netherlands).

Microbiological analysis

Samples (10 g) from inoculated meat in the presence of competitive microflora were weighed aseptically, added to

sterile quarter-strength Ringer's solution (90 ml) and homogenized in a stomacher (Lab Blender 400; Seward Medical, London, UK) for 60 s at room temperature. Decimal dilutions in quarter-strength Ringer's solution were prepared and duplicate 1- or 0·1-ml samples of appropriate dilutions poured or spread on the following media: Plate Count Agar (PCA; 1.05463; Merck) for total viable count (TVC), incubated at 25 °C for 72 h; *B. thermosphacta* on Gardner's (1966) STAA medium supplemented with streptomycin sulphate, thallous acetate and cycloheximide (actidione; this medium was made from basic ingredients in the laboratory and incubated at 25 °C for 72 h); MRS (1.10660; Merck) for lactic acid bacteria, overlaid with the same medium and incubated at 25 °C for 96 h under anaerobic conditions; *Pseudomonas* spp. on cetrimide-fucidin-cephaloridine agar (CM559 supplemented with selective supplement SR 103E; Oxoid, Basingstoke, UK) incubated at 25 °C for 48 h; yeasts on Rose Bengal Chloramphenicol Agar (supplemented with chloramphenicol supplement X009; Lab M 36) incubated at 25 °C for 5 d; Enterobacteriaceae on Violet Red Bile Dextrose Agar (1.10275; Merck) incubated at 37 °C for 24 h; and *L. monocytogenes* on Palcam-Listeria Selective (PLS) Agar (1.11755 supplemented with Palcam Listeria Selective Supplement 1.12122; Merck).

The enumeration of the inoculated population in meat samples without the competitive microflora was performed by using PLS agar as described above. In order to check the sterility of meat fillets TVC were determined using PCA. In cases where colonies of *L. monocytogenes* were not evident on the agar plates from the 10-fold dilution, an enrichment technique was used for the resuscitation of possibly injured living cells as follows: 10 g meat sample was suspended in 500 ml Selenite Cystine enrichment broth (1.07709; Merck) and incubated at 35 °C for 12–18 h. One ml of the above medium was then serially diluted and spread on PLS plates.

Experimental design

A three-way analysis of variance experiment was designed. The development of spoilage microflora as well as the growth/survival of *L. monocytogenes* were evaluated under three gaseous atmospheres (air, vacuum and 40% CO_2/30% O_2/30% N_2), two values of O_2 permeability (3600 and 4·5 $cm^3\ m^{-2}\ 24\ h^{-1}$) and two concentrations of oregano essential oil (0% and 0·8% v/w) on meat with or without (sterile) background flora and stored at 5 °C. This procedure was performed twice and duplicate samples for each treatment were taken. The growth data from plate counts were transformed to $\log_{10}$ values. The Baranyi model (Baranyi *et al.* 1993) was fitted to the logarithm of the viable cell concentration. For curve fitting, the in-house program DMFit (Institute of Food Research, Reading, UK) was used, which was kindly provided by Dr J. Baranyi.

RESULTS

Development of autochthonous flora

The microbial association of meat consisted of *Pseudomonas* spp., lactic acid bacteria and *B. thermosphacta* (Figs 1–5). It needs to be stressed that the contribution of each group in the consortium, as well as their rate of growth, lag phase

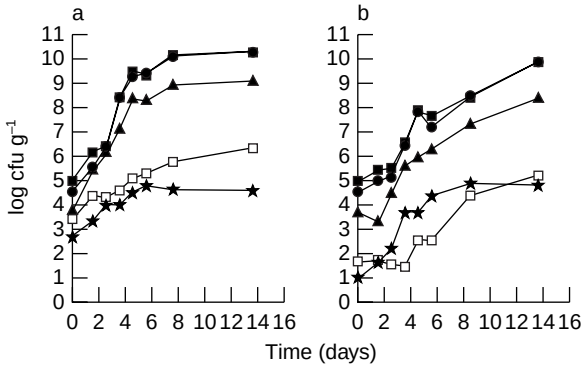

Fig. 1 Growth of *Listeria monocytogenes and* competitive microflora on naturally contaminated meat with (a) 0% and (b) 0·8% oregano essential oil, stored in air at 5 °C. □, *Listeria monocytogenes*; ■, total viable count; ●, pseudomonads; ▲, *Brochothrix thermosphacta*; ★, lactic acid bacteria. Each number is the mean of two samples taken from different experiments. Each sample was analysed in duplicate (coefficient of variation < 5%)

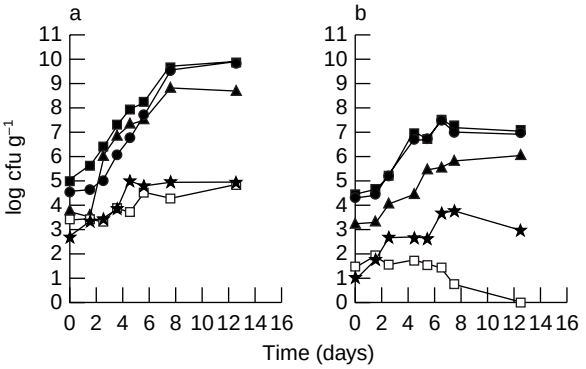

Fig. 2 Growth/survival of *Listeria monocytogenes and* competitive microflora on naturally contaminated meat with (a) 0% and (b) 0·8% oregano essential oil, packaged in 40% CO_2/30% O_2/30% N_2 within high-permeability film at 5 °C. □, *Listeria monocytogenes*; ■, total viable count; ●, pseudomonads; ▲, *Brochothrix thermosphacta*; ★, lactic acid bacteria. Each number is the mean of two samples taken from different experiments. Each sample was analysed in duplicate (coefficient of variation < 5%)

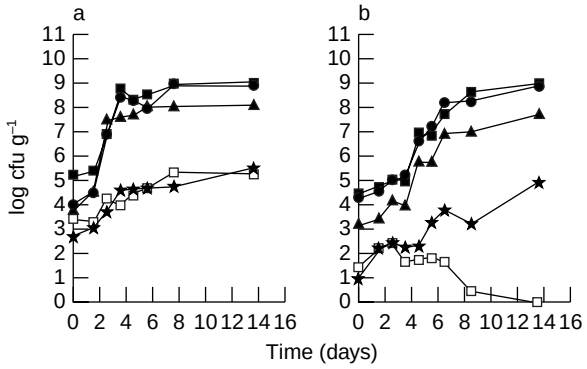

Fig. 3 Growth/survival of *Listeria monocytogenes and* competitive microflora on naturally contaminated meat with (a) 0% and (b) 0·8% oregano essential oil, packaged in vacuum within high-permeability film at 5 °C. □, *Listeria monocytogenes*; ■, total viable count; ●, pseudomonads; ▲, *Brochothrix thermosphacta*; ★, lactic acid bacteria. Each number is the mean of two samples taken from different experiments. Each sample was analysed in duplicate (coefficient of variation < 5%)

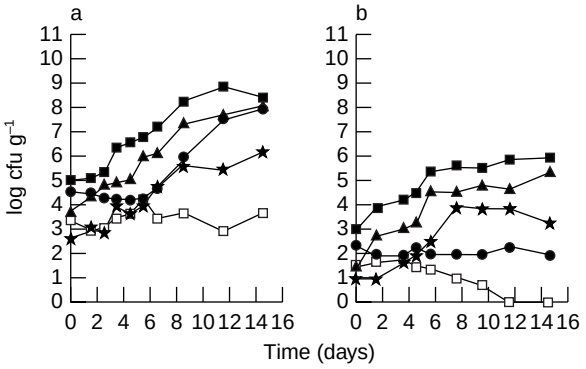

Fig. 5 Growth/survival of *Listeria monocytogenes and* competitive microflora on naturally contaminated meat with (a) 0% and (b) 0·8% oregano essential oil, packaged in vacuum within low-permeabilty film at 5 °C. □, *Listeria monocytogenes*; ■, total viable count; ●, pseudomonads; ▲, *Brochothrix thermosphacta*; ★, lactic acid bacteria. Each number is the mean of two samples taken from different experiments. Each sample was analysed in duplicate (coefficient of variation < 5%)

and final counts, was affected by the packaging conditions and the permeability of the packaging film as well as by the addition of oregano essential oil (Table 1). Indeed, in samples stored under aerobic conditions, pseudomonads predominated by yielding maximum levels of 10 $\log_{10}$ cfu g^{-1} followed by *B. thermosphacta*, while lactic acid bacteria increased by only 2 $\log_{10}$ cfu g^{-1} (Fig. 1a; Table 1). Modified atmosphere packaging (40% CO_2/30% O_2/30% N_2) and VP of meat suppressed the maximum level of aerobic counts compared with aerobic storage (Figs 2a–5a). However, when the high-permeability film was used, the spoilage pattern was similar to that of aerobic storage (Table 1; Figs 2a and 3a). *Brochothrix thermosphacta*, however, was the dominant organism in VP/MAP conditions using low-permeability film (Figs 4a and 5a). Practically, no growth was evident for pseudomonads in MAP (Fig. 4a), while an increase of 4 $\log_{10}$ cfu g^{-1} occurred after a delay of 6 d in VP (Table 1; Fig. 5a). The contribution of yeasts and Enterobacteriaceae was insignificant since they did not grow during the storage of meat (results not shown).

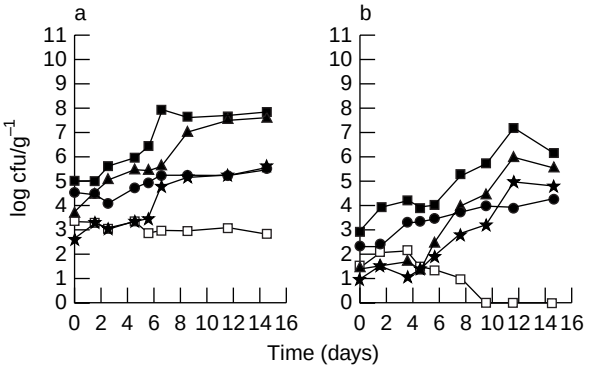

Fig. 4 Growth/survival of *Listeria monocytogenes* and competitive microflora on naturally contaminated meat with (a) 0% and (b) 0·8% oregano essential oil, packaged in 40% CO_2/30% O_2/30% N_2 within low permeability film at 5 °C. □, *Listeria monocytogenes*; ■, total viable count; ●, pseudomonads; ▲, *Brochothrix thermosphacta*; ★, lactic acid bacteria. Each number is the mean of two samples taken from different experiments. Each sample was analysed in duplicate (coefficient of variation < 5%)

The addition of 0·8% (v/w) oregano essential oil, in general, resulted in an initial reduction of 2–3 $\log_{10}$ in the members of the microbial association regardless of the packaging film. In fact, the Gram-negative pseudomonads seemed to be the group most resistant to oregano essential oil compared with the other spoilage flora. Lactic acid bacteria were the most sensitive group in all cases (Figs 1b–5b) and, although their initial population was reduced, their growth rates were greater than air or similar to VP/MAP identically-treated samples without the addition of oregano essential oil (Table 1). The inhibitory effect of oregano essential oil on the spoilage flora was similar in both aerobic and gaseous environments within high-permeability film (Figs 1b–3b). Indeed, despite the low yield in counts, pseudomonads predominated under aerobic conditions as

Table 1 The effect of packaging in air, vacuum (VP) and 40% CO_2/30% O_2/30% N_2 modified atmosphere (MAP) using high (H) and low (L) O_2-permeable film and the addition of oregano essential oil on the final population, lag period and maximum specific growth rate of *Listeria monocytogenes* and competitive microflora on meat stored at 5 °C (three way analysis of variance)

		Addition of oregano essential oil*					
		Without			With		
Pack*	Micro-organism*	Final population ($\log_{10}$ cfu g^{-1})	Lag (d)	Rate (d^{-1})	Final population ($\log_{10}$ cfu g^{-1})	Lag (d)	Rate (d^{-1})
Air	*L. monocytogenes*	6·37 ± 0·18¶	0	0·31 ± 0·03	5·30 ± 0·27	3·44 ± 0·62	0·56 ± 0·09
	Pseudomonads	10·02 ± 0·25	0	1·28 ± 0·18	9·86 ± 0·59	0	0·53 ± 0·09
	Br. thermosphacta	8·87 ± 0·16	0	0·97 ± 0·07	8·33 ± 0·50	1·52 ± 0·44	0·52 ± 0·08
	Lactic acid bacteria	4·66 ± 0·09	0	0·41 ± 0·05	4·90 ± 0·21	0	0·68 ± 0·08
MAP (H)	*L. monocytogenes*	4·84 ± 0·16	0	0·13 ± 0·02	1	4·66 ± 1·10	− 0·23 ± 0·04
	Pseudomonads	9·88 ± 0·11	2·11 ± 0·16	0·94 ± 0·04	7·11 ± 0·14	1·95 ± 0·55	0·77 ± 0·16
	Br. thermosphacta	8·69 ± 0·46	0·61 ± 0·32	0·86 ± 0·15	6·02 ± 0·23	1·02 ± 0·54	0·41 ± 0·05
	Lactic acid bacteria	4·96 ± 0·20	0	0·43 ± 0·08	3·45 ± 0·33	0	0·35 ± 0·12
MAP (L)	*L. monocytogenes*	2·84 ± 0·13	0	− 0·03 ± 0·01	1	3·95 ± 1·25	− 0·31 ± 0·01
	Pseudomonads	5·41 ± 0·11	3·34 ± 1·39	0·29 ± 0·17	4·66 ± 0·25	1·51 ± 0·34	0·25 ± 0·05
	Br. thermosphacta	7·67 ± 0·32	0	0·33 ± 0·04	5·79 ± 0·35	4·28 ± 0·86	0·66 ± 0·13
	Lactic acid bacteria	5·38 ± 0·13	4·23 ± 0·57	0·47 ± 0·27	4·85 ± 0·37	4·24 ± 0·99	0·48 ± 0·09
VP (H)	*L. monocytogenes*	5·36 ± 0·26	0	0·28 ± 0·05	1	4·16 ± 1·85	− 0·23 ± 0·06
	Pseudomonads	8·91 ± 0·16	0·78 ± 0·41	0·97 ± 0·13	8·66 ± 0·22	2·66 ± 0·48	0·99 ± 0·17
	Br. thermosphacta	8·14 ± 0·16	0	0·94 ± 0·22	7·70 ± 0·45	0	0·56 ± 0·08
	Lactic acid bacteria	5·17 ± 0·22	0	0·45 ± 0·08	4·98 ± 0·19	0	0·51 ± 0·06
VP (L)	*L. monocytogenes*	3·69 ± 0·13	3·51 ± 1·01	0·02 ± 0·01	1	4·87 ± 1·07	− 0·23 ± 0·05
	Pseudomonads	7·98 ± 0·16	6·12 ± 0·45	0·65 ± 0·09	2·00 ± 0·16	0	0
	Br. thermosphacta	8·09 ± 0·23	0	0·41 ± 0·03	5·11 ± 0·22	0	0·48 ± 0·09
	Lactic acid bacteria	6·20 ± 0·32	3·12 ± 0·54	0·35 ± 0·03	3·75 ± 0·16	2·69 ± 0·73	0·53 ± 0·18

*Each factor and their interactions are significant at the level of 99·99%.
¶S.D.

well as in VP/MAP sealed with the high-permeability film. However, the use of low O_2-permeable film in VP/MAP enhanced the inhibitory effect of oregano essential oil on the microbial association (Figs 4b and 5b). For example in VP/MAP, the combination of 0·8% oregano essential oil and film of low O_2-permeability totally prevented the growth of pseudomonads in comparison with control samples. In this case, *B. thermosphacta* was found to be the major contributor to the total flora (Fig. 5b).

Growth of *Listeria monocytogenes* in the presence or absence of background flora

Listeria monocytogenes was inoculated on meat fillets either with or without natural contaminating microflora in order to evaluate the effect of competitive background flora on its growth. Prior to inoculation, the presence of *L. monocytogenes* was tested and this pathogen was not detected. Moreover, there was no detectable contamination of sterile meat throughout the storage period. At each sampling time, the counts of *L. monocytogenes* on selective agar were in agreement with the counts on PCA (results not shown). The presence of background flora affected the growth/survival of this pathogen. Indeed, growth of this pathogen was evident in naturally contaminated samples stored under aerobic or VP/MAP using high-permeability film (Figs 1a–3a). However, under identical storage conditions, there was a limited growth of the pathogen on sterile fillets (Fig. 6). It should be noted that the use of low-permeability film in both sterile and naturally contaminated fillets controlled the growth of *L. monocytogenes* throughout the storage period, regardless of the gaseous atmospheres (Figs 4a, 5a and 6).

The addition of 0·8% (v/w) oregano essential oil to samples with background flora affected the initial counts as well as the growth of *L. monocytogenes* (Figs 1b–5b). Indeed, in all samples there was an initial reduction of

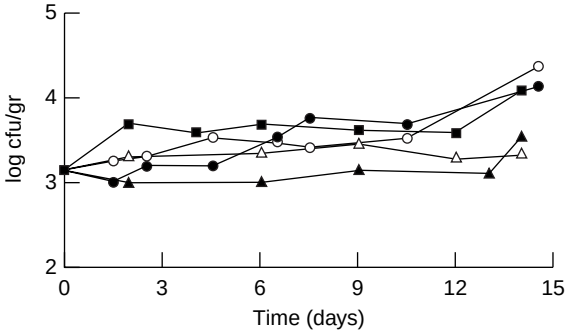

Fig. 6 Growth/survival of *Listeria monocytogenes* on sterile meat packaged in air (■), 40% CO_2/30% O_2/30% N_2 (open symbols) and vacuum (solid symbols) within high permeabilty (○, ●) and low-permeabilty film (△, ▲) at 5 °C. Each number is the mean of two samples taken from different experiments. Each sample was analysed in duplicate (coefficient of variation < 5%)

inoculum of about 1 log cfu units. Subsequently, this pathogen grew only in samples stored aerobically (Fig. 1b), while a decline in counts was evident in samples stored under VP/MAP conditions regardless of the film permeability (Figs 2b–5b). The numbers of *L. monocytogenes* on sterile fillets decreased to undetectable levels, even after resuscitation, at very early stages of storage under all conditions tested (results not shown).

DISCUSSION

The importance of principles of food microbial ecology in understanding the growth/survival of micro-organisms in foods is well documented (Body and Wimpenny 1992). Many of these principles have been used for the development of new technologies or strategies to control either spoilage or food-borne outbreaks (e.g. MAP, natural antimicrobial systems, predictive microbiology and risk analysis). For example, this is the case with *L. monocytogenes* where various parameters, either intrinsic (pH, a_w, redox potential and available nutrients) or extrinsic (the ratio of O_2/CO_2, temperature and addition of chemical or biopreservatives), have been tested to control or predict the growth of this particular pathogen (Chen and Shelef 1992; Grau and Vanderlinde 1992; Harmayani *et al.* 1993; Wijtzes *et al.* 1993; Farber *et al.* 1996; George *et al.* 1996). It needs to be noted, however, that the literature contains conflicting reports on the ability of the above-mentioned hurdles (e.g. VP/MAP) to control *L. monocytogenes.* In such systems emphasis has been given to the concentration of CO_2 rather than to the ability of the film used to maintain the flushed gaseous composition (Stanbridge and Davies 1998). Since almost all packaging film materials available so far permit some O_2 penetration, it is not surprising that controversial results have been reported for the inhibition of *L. monocytogenes* by VP/MAP. For example, growth of *L. monocytogenes* was detected in VP/MAP beef at 5 °C using film of O_2 transmission rate 12 g m^{-2} 24 h^{-1} at 20 °C and 85% r.h. (Hudson and Mott 1993), beef at 3 °C within a film of 2 g m^{-2} 24 h^{-1} at 20 °C and 85% r.h. (Hudson *et al.* 1994), lamb at 5 °C within a film of 8 cm^3 m^{-2} 24 h^{-1} at 25 °C and 50% r.h. (Sheridan *et al.* 1995), chicken breast at 7 °C within a polyamide/polyethylene film (Beumer *et al.* 1996) and poultry at 3·5, 6·5 and 10 °C within a film of 40 cm^3 m^{-2} 24 h^{-1} at 23 °C and 0% r.h. (Barakat and Harris 1999). However, no growth was observed in packaged lamb at 0 and 5 °C in vacuum using a film of 48 cm^3 m^{-2} 24 h^{-1} at 25 °C and 50% r.h. and at 0 °C in different modified atmospheres within a film of 8 cm^3 m^{-2} 24 h^{-1} at 25 °C and 50% r.h. (Sheridan *et al.* 1995). In our study, either no or limited growth of *L. monocytogenes* occurred only in meat samples stored in VP/MAP within O_2-impermeable film either in the presence or absence of background flora (Figs 4–6). However, there was growth of *L. monocytogenes* in meat samples packaged either under aerobic conditions or with VP/MAP using high permeability film (Figs 1–3). Limited growth of this pathogen was observed in sterile meat incubated under identical storage conditions (Fig. 6). In O_2-permeable packs, the dramatic changes in the composition of the gaseous atmosphere within VP/MAP (McMullen and Stiles 1991) enhanced growth of pseudomonads (Newton and Rigg 1979) and, as a consequence, stimulation of *L. monocytogenes* can occur. Similar observations have been reported for beef, milk and precooked chicken nuggets (Gouet *et al.* 1978; Marshall and Schmidt 1988; Farrag and Marth 1989; Marshall *et al.* 1992). The hydrolysis of proteins, which could provide free amino acids, has been considered as a likely explanation for the stimulus of *L. monocytogenes* growth by pseudomonads in the case of milk (Marshall and Schmidt 1991). Proteolysis caused by the microbial association and *Pseudomonas fragi* was also evident in chicken breast stored under aerobic, VP and MAP conditions (Nychas and Tassou 1997). The fact that pseudomonads did not release such nutrients on endive leaves could be the reason for the lack of stimulation of *L. monocytogenes* (Carlin *et al.* 1996). Furthermore, inhibition of *L. monocytogenes* by pseudomonads in a sterile minced beef medium was ascribed to competition for nutrients (Mattila-Sandholm and Skyttä 1991).

Other members of the meat microbial association and/or their metabolic end-products could also influence the growth of *L. monocytogenes. (*Thomas *et al.* 1997; Nychas *et al.* 1998). Indeed, in VP/MAP there is a shift from a Gram-negative aerobic flora towards Gram-positive bac-

teria, comprised mainly of lactic acid bacteria and/or *B. thermosphacta* (Nychas *et al*. 1998). This was evident in our study in meat samples stored under vacuum or MA packaging within the low-permeability film. However, an increase in pseudomonads in vacuum of *ca* 1000-fold (Fig. 5a) has also been reported (Nissen *et al*. 1996). The variability of ecological determinants (O_2 tension, type of available substrate and temperature) strongly affects the end-products formation by lactic acid bacteria and *B. thermosphacta* (Nychas *et al*. 1998). Bacteriocins, H_2O_2, lactate, acetate and formate are among these products, which could also contribute further to inhibition of *L. monocytogenes* (Campanini *et al*. 1993; Jeppesen and Huss 1993; Östling and Lindgren 1993; Muriana 1996; Thomas *et al*. 1997). However, the effect of the background flora on the inhibition of *L. monocytogenes* is in dispute (Jeong and Frank 1994; Beumer *et al*. 1996; Francis and O'Beirne 1998; Barakat and Harris 1999; Buchanan and Bagi 1999). This could be due to the fact that much of this knowledge is derived from laboratory media that are incapable of simulating the unique situation of a 'real' environment in which there is diversity among the members of the microbial association as well as their physiological status. A similar situation concerning the inhibitory 'performance' of natural antimicrobial compounds is also evident. In fact, the greater inhibitory effect of oregano essential oil against food-borne spoilage bacteria and moulds that have been reported extensively in broths (Paster *et al*. 1990, 1995; Daouk *et al*. 1995; Quattara *et al*. 1997) in comparison to solid foods can be attributed to the different food structure (Tassou *et al*. 1996; Skandamis and Nychas 2000; Skandamis *et al*. 2000).

Indeed, bacterial growth in liquid medium occurs planktonically, while either on or within a solid matrix, bacteria form discrete colonies (Robins *et al*. 1994). In the latter case, the cells are immobilized and localized in high densities in the food matrix, whilst little is known about the specific biochemical and physiological properties of such growing bacteria. Challenge tests in model systems simulating the food matrix revealed that the properties of bacterial growth or survival, tolerance to extremes and even biochemical activity can be significantly different from those of cells growing freely in liquid cultures (Brocklehurst *et al*. 1997; Skandamis *et al*. 2000). These differences can be due to the population density *per se*, diffusivity and availability, competition for nutrients (e.g. glucose), O_2 affinity and accumulation of end-products (Stecchini *et al*. 1993; Thomas *et al*. 1997; Skandamis *et al*. 2000). Consequently, the application of identical ecological determinants (e.g. pH, a_w, redox potential, packaging, antimicrobial concentration, etc.) may have different effects (e.g. rate of growth, consumption of available carbon sources, type and rate of metabolic end-products) on the same bacteria when acting in different food ecosystems.

In this study the addition of oregano essential oil significantly influenced both *L. monocytogenes* and spoilage flora (Figs 1b–5b). It should be noted that *L. monocytogenes* was eliminated, whereas only a selective inhibition of the background flora of beef occurred under all packaging conditions. The inhibition of this pathogen under aerobic conditions with various essential oils (e.g. oregano, rosemary, eugenol and pimento) has also been reported in 'sterile' liver sausage and in cooked poultry (Pandit and Shelef 1994; Hao *et al*. 1998). Despite these studies performed in the 'absence' of background flora, there is a lack of information related to the inhibitory effect of essential oils in naturally contaminated foods (e.g. fish) stored in combination with VP/MAP conditions (Tassou *et al*. 1996). In this study data are provided for fresh meat. The limitations to the practical use of essential oils in foods may also be attributed to their flavouring characteristics and the need for relatively high effective concentrations compared with culture media, for the reasons discussed above. With respect to meat preservation, the selection of packaging conditions indirectly affects the inhibitory potential of essential oils. For instance, in the present study, oregano essential oil was less effective in aerobically stored samples and those packed under VP/MAP within the high-permeability film (Figs 1b–3b) in comparison with samples stored within low-permeability film (Figs 4b and 5b). In this case not only did a significant inhibition occur but also qualitative changes in the microbial association were evident. Indeed lactic acid bacteria eventually outgrew pseudomonads compared with the untreated samples (Table 1; Figs 4 and 5). These qualitative changes among the members of the association result from the combined action of the modified atmosphere and oregano essential oil. Thus the use of VP/MAP in conjunction with oregano essential can be seen as a means of reducing the effective dose of the latter in foods, since the combined hurdles may have a synergistic effect. A similar study has been performed using mint essential oil (Tassou *et al*. 1995). Further research is needed in this field.

ACKNOWLEDGEMENTS

This research was funded by EU (DGXII), project FAIR-ct-1066. P. Skandamis would like to thank the Greek Scholarship Foundation.

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