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Growth characteristics of *Listeria monocytogenes*, *Listeria welshimeri* and *Listeria innocua* strains in broth cultures and a sliced bologna-type product at 4 and 7 °C

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Abstract

The growth characteristics of meat processing plant-derived field strains of *Listeria monocytogenes*, *L. welshimeri* and *L. innocua* were analyzed. The strains were inoculated in BHI broth cultures and incubated at 4 and 7 °C. Growth curves were determined by colony counting for 28 days. Significant variations were detected in the growth properties of these field-derived strains. In particular some of the *L. monocytogenes* strains displayed better cold stress tolerance. These discrepancies in growth behavior were more apparent in the cultures at 4 °C compared to 7 °C. Similar growth characteristics were observed for selected *L. monocytogenes* strains also in food challenge tests based on a sliced bologna-type product. The results stress the need for more evaluation of field strain growth characteristics and incorporation of such information in relevant predictive microbial growth models for *L. monocytogenes* risk assessment in naturally contaminated food products.

Keywords: *Listeria* spp.; Growth characteristics; 4 °C; 7 °C

1. Introduction

The bacteria of the genus *Listeria* are widely distributed in nature as well as in different food processing and storage environments. *Listeria monocytogenes* as a food-borne pathogen has significant public health and economic impacts. The infection of humans may result in severe clinical disease as well as high mortality (Posfay-Barbe and Wald, 2004). Many food products have been implicated as routes for human infection (Farber and Peterkin, 1991; Rocourt et al., 2000). Furthermore *L. monocytogenes* contamination is one of the leading causes of recalls in industrially processed foods due to microbiological safety concerns. The *Listeria* spp. are psychrotolerant and have the capacity to grow from 1 to 45 °C, but their optimal growth temperatures range from 30 to 37 °C (Wilkins et al., 1972; Seeliger and Jones, 1986). The ability to proliferate at low environmental temperatures obviously presents a major challenge to food safety with regard to *L. monocytogenes*. These foodborne pathogens persist in food processing environments and proliferate during storage of chilled food products.

One aspect of risk assessment of *L. monocytogenes* in food products depends on the application of predictive microbiology growth models (Soboleva et al., 2000). The generation and application of such predictive models relies on knowledge about the growth characteristics of *L. monocytogenes* under different environmental parameters. Important parameters in this regard include the type of food matrix, storage temperature conditions, water phase salt/water activity and pH, as well as other food environment-related intrinsic and extrinsic factors. Along these lines several *L. monocytogenes* predictive growth models have been developed and evaluated (Koutsoumanis and Sofos, 2005; Lu et al., 2005; Francois et al., 2006). *L. monocytogenes* strain diversity with regard to the organism's response to food processing and storage-related environment challenges is also another factor that influences the predictive power of microbial growth models

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applied in risk assessment. Available data from literature indicates variable responses in different field isolates of *L. monocytogenes* with regard to food environment-related stress factors such as reduced growth temperatures, osmotic stress and low pH (Barbosa et al., 1994; Liu et al., 2005). As a consequence, the current predictive models if based on a few reference strains or field isolates may not adequately address these aspects in view of *L. monocytogenes* risk assessment. Therefore, an improved understanding of the relationship between *L. monocytogenes* field strain diversity and growth responses under food environment-related stress is important to allow more realistic predictions of risk, associated with different *L. monocytogenes* strains that may contaminate food products.

The aim of the present study was to evaluate growth characteristics of a collection of *Listeria* spp. strains that includes *L. monocytogenes*, *L. innocua* and *L. welshimeri* derived from an industrial meat processing plant. The growth behavior of these organisms was investigated in BHI cultures during incubation at 4 and 7 °C. Furthermore, to assess the growth potential of two different *L. monocytogenes* strains in food environments, challenge tests based on a normal recipe and a potassium lactate treated bologna-type product were also performed.

2. Materials and methods

2.1. Bacterial strains and their molecular characterizations

The different *Listeria* spp. strains used in this study were isolated from an industrial meat processing plant and are listed in Table 1. Serotyping of the *L. monocytogenes* and *L. innocua* was done at the Swiss National Centre for Listeriosis (Lausanne, Switzerland). For further molecular strain characterization DNA templates were prepared from stationary cultures using the Qiagen tissue DNA extraction kit (Qiagen AG, Hombrechtikon, Switzerland). The lineage divisions of the different *L. monocytogenes* strains was determined on their respective DNA templates using the ASO-PCR multiplex system based on the *prfA* virulence gene cluster as previously described (Ward et al., 2004). The ERIC and REP rep-PCR reactions were performed as previously described (Harvey et al., 2004). The REP primers used were REP 1R-I (5′-IIICGICGICATCI GGC-3′) and REP2-I (5′-ICGICTTATCIGGCCTAC-3′). The ERIC primers used were ERIC 1R (5′-ATGTAAG CTCCTGGGGATTCAC-3′) and ERIC 2 (5′-AAGTAA GTGACTGGGGT). Amplification reactions were carried out in 50 µl reaction mixtures containing 100 ng of DNA template, 50 pmol of each of the two opposing primers (REP 1R-I/REP 2-I or ERIC 1R/ERIC 2; Microsynth AG), 200 µM each dNTP, 2.5 U *Taq* DNA polymerase (Promega, USA), and 1 × polymerase buffer provided by the supplier (Promega) with a final $MgCl_2$ concentration adjusted to 2.5 mM. The amplifications were performed in a T3 cycler using the temperature profiles previously described by Jersek et al. (1996) for rep-PCR. The products were separated on 2% agarose gels, stained with ethidium bromide and visualized under UV light.

Table 1
Listeria species strains used in this study

Strain designation	Serotype	Source
L. monocytogenes		
L.m. 22/3A	1/2a	Asymptomatic human carrier
L.m. 25/9	1/2c	Process line
L.m. 156/2A	1/2a	Meat sample
L.m. 217	1/2a	Process line
L.m. 288	1/2a	Process line
L.m. 760	1/2c	Process line
L. welshimeri		
L.w. 24/4	—	Process line
L.w. 517/9	—	Process line
L.w. 12/00/9	—	Process line
L.w. 173/3	—	Process line
L.w. 223/A	—	Meat sample
L.w. 485/3	—	Process line
L. innocua		
L.i. 52/1	6a	Process line
L.i. 12/00/10	6a	Process line
L.i. 191/GNZ	6a	Meat sample
L.i. 600/3	6a	Process line
L.i. 573/2A	6a	Meat sample

2.2. Preparation of inocula

The strains were available as frozen (−70 °C) stock cultures in tryptic soy broth (TSB) (Becton and Dickenson, Sparks, USA) supplemented with 20% glycerol. They were revived by plating out an aliquot onto columbia agar plates supplemented with 5% sheep blood (Becton and Dickenson) and incubating overnight at 37 °C. Single colonies were picked on the next day and inoculated into 10 ml brain heart infusion broth (BHI) (Oxoid, Hampshire, UK) followed by an incubation for 20 h at 37 °C. We confirmed by colony counting that this procedure gave rise to stationary phase cultures containing 9.0 log cfu/ml for the different *Listeria* isolates and their species lab control strains.

2.3. Evaluation of growth behavior in BHI broth cultures

The standardized inocula prepared as outlined above were 10-fold serially diluted in fresh BHI broth to give 10 ml broth cultures containing approximately 10^{-1}, 10^{0}, 10^{1} and 10^{2} cfu/ml. The BHI growth evaluation experiments for each *Listeria* spp. were prepared in two sets of triplicates. Subsequently one set of the sample triplicates was incubated at 4 °C and the other set at 7 °C. The growth kinetics for the sample triplicates were assessed on a weekly basis at days 0, 7, 14, 21 and 28 using standard colony counting procedures as described below. The results presented in this study therefore reflect average counts