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Short communication

Lactic acid bacteria in dried vegetables and spices

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ABSTRACT

Spices and dried vegetable seasonings are potential sources of bacterial contamination for foods. However, little is known about lactic acid bacteria (LAB) in spices and dried vegetables, even though certain LAB may cause food spoilage. In this study, we enumerated LAB in 104 spices and dried vegetables products aimed for the food manufacturing industry. The products were obtained from a spice wholesaler operating in Finland, and were sampled during a one-year period. We picked isolates ($n = 343$) for species identification based on numerical analysis of their ribotyping patterns and comparing them with the corresponding patterns of LAB type strains. We found LAB at levels >2 log CFU/g in 68 (65%) of the samples, with the highest counts detected from dried onion products and garlic powder with counts ranging from 4.24 to 6.64 log CFU/g. The LAB identified were predominantly *Weissella* spp. (61%) and *Pediococcus* spp. (15%) with *Weissella confusa*, *Weissella cibaria*, *Weissella paramesenteroides*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* being the species identified. Other species identified belonged to the genera of *Enterococcus* spp. (8%), *Leuconostoc* spp. (6%) and *Lactobacillus* spp. (2%). Among the LAB identified, *Leuconostoc citreum*, *Leuconostoc mesenteroides* and *W. confusa* have been associated with food spoilage. Our findings suggest that spices and dried vegetables are potential sources of LAB contamination in the food industry.

1. Introduction

Spices are often defined as aromatic, dried plant substances applied to foods primarily for flavoring and coloring, rather than for nutritional values. In addition to true spices, such as black pepper, the term spice is often used in a wider context to include dried herbs, aromatic seeds and dried vegetables, such as onion and garlic (Coggins, 2001). Similarly with other agricultural commodities, spices are exposed to microbial contamination from various sources including soil, insects, and irrigation water. Subsequently, spices may harbor a diverse and abundant microbial community, and carry a risk of introducing harmful microbes into the food production chain.

Unless given a specific decontamination treatment, spices may contain microbial counts as high as 10^7–10^8 CFU/g (Coggins, 2001). In the food industry, such contamination is significant for two main reasons: firstly, harmful microbes from the spices may cause health hazards or spoilage in foods which do not undergo further processing; and secondly, harmful microbes originating from spices may spread throughout the processing environment and lead to post-process contamination of finished products. To reduce or eliminate microbes in spices and dried vegetables, many spices must be given an antimicrobial treatment. Gamma irradiation is an effective process reducing microbial numbers and growth, and is used in some countries. However, in many European countries, irradiated food lacks a wide consumer acceptance, and thus, most food business operators reject to use irradiated spices or foods containing them (Farkas and Mohácsi-Farkas, 2011). Furthermore the process of certain spices and vegetables products involves a heat or steam treatment to reduce microbial numbers. However, thermal treatments are not applicable to all products, such as dried onion and garlic, which easily lose their characteristic sensory or functional properties during thermal treatment. Due to lack of effective and commercially acceptable decontamination methods, dried onion and garlic products often enter the food production chain “untreated”, with their microbiological quality relying on control measures and hygiene practices taken during pre- and post-harvest, and washing, cutting and drying operations.

The meat industry is a large and important consumer of spices, herbs and dried vegetable seasonings. These commodities are used to season cured and processed meats, but also added to flavor raw

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meat preparations. In the meat industry, spices and dried vegetables containing high loads of microbes are considered a risk for spoilage bacterial contamination both directly and indirectly via contaminated ingredients, surfaces and processing line (Cerveny et al., 2010; Coggins, 2001). Whilst the heat treatment of cured-meat products is usually enough to significantly reduce the numbers of vegetative spoilage bacteria, this is not the case with seasoned raw meat preparations, such as marinated meats and hamburger patties. The shelf life of such raw meat products is often limited by psychrotrophic lactic acid bacteria (LAB) growing and causing deteriorations (Nieminen et al., 2012; Susiluoto, 2003). The fact that plants and vegetables are natural habitats for many LAB, has raised concerns if spices and dried vegetables are sources of spoilage LAB contamination in the meat chain. However, little is known about LAB on spices and dry vegetables, and in particular, if spices may introduce spoilage LAB into the meat processing chain.

The present study was initiated in response to meat processors and spice suppliers concerns discussed above, and aimed to enumerate LAB and to identify the predominant species in spices and dried vegetables used in the meat and meal industry. The isolation procedure focused on psychrotrophic, heterofermentative LAB, such as *Lactobacillus* spp., *Leuconostoc* spp., and *Weissella* spp., a group of LAB associated with spoilage of marinated meats (Koort et al., 2005; Nieminen et al., 2012; Susiluoto, 2003) and cured-meat products (Koort et al., 2006; Samelis, 2006; Santos et al., 2005).

2. Materials and methods

2.1. Sampling

Batches of spice and dried vegetables aimed for the food industry were supplied and sampled (approximately 200–400 g) during a one-year period by a spice distributor operating in Finland. The sampling was focused on dried onion products, which according to the collaborating spice distributor, frequently showed high numbers of aerobic mesophilic microbes often exceeding the limit of 10^6 CFU/g specified in the purchase agreement. Product details including those on treatment or decontamination methods, were recorded from the product specification sheets obtained from the collaborating distributor. Furthermore, the colony counts for mesophilic aerobic microbes (aerobic plate counts, APC) had been determined on Plate Count Agar at 30 °C as part of the spices distributor's own quality control program. Products, number of samples included, and the mean APC for each product included are listed in Table 1. The dried onion products sampled had been produced and processed in India. Other products had been processed either in France, Germany, the Netherlands or Spain, but for most products, the harvesting country of the produce was unknown.

2.2. Enumeration of LAB

A sample of 25 g of each batch was homogenized with 225 ml of saline peptone (0.1%) water by a stomacher, and the sample homogenates were decimally diluted in saline peptone water. Plate count for LAB was determined by plating 100 μL of appropriate dilutions onto MRS agar (pH 6.2; Oxoid, United Kingdom) supplemented with amphotericin B (Sigma–Aldrich, USA) at 10 mg/l to reduce mold growth (NMKL method No. 140; NMKL, 2007). Plates were incubated at 25 °C for 4–5 days in sealed jars in an anaerobic, carbon dioxide-rich atmosphere created with AnaeroGen sachets (Oxoid). All colonies that were visible after 4–5 days h were counted, and included in the LAB count.

2.3. Identification of LAB

2.3.1. HindIII ribotyping with 16S and 23S rRNA gene targeting probes

For each sample with LAB counts exceeding 3.2 log CFU/g, 6–10 colonies were picked, and subcultured at 25 °C in MRS broth (Difco) and on MRS agar. Ribotyping was performed as described previously (Vihavainen et al., 2008) using five probes (Regnault et al., 1997) targeting the 16S and 23S rRNA encoding genes. Briefly, the cells from 1.5 ml of MRS broth were pelleted by centrifugation, the total DNA was extracted and fragmented with HindIII restriction endonuclease, the resulting DNA fragments were resolved by agarose gel electrophoresis, and blotted onto a nylon membrane. Southern blots were hybridized with digoxigenin-labeled probes,

Table 1
Mean counts (log CFU/g) of aerobic mesophilic microbes (APC) and lactic acid bacteria (LAB), and distribution of counts for LAB in spices and dried vegetables.

Product (*n*)	APC[a]	LAB	Range of count[b] (log CFU/g)			
			<2.00	2.00–4.00	4.01–6.00	>6.00
Air dried						
Garlic granules (5)	4.82 ± 0.29[c]	2.41 ± 0.47		5		
Garlic powder (5)	4.97 ± 0.23	4.88 ± 0.64			5	
Onion granules (9)	5.40 ± 0.28	4.91 ± 0.55		1	8	
Onion flakes (5)	4.40 ± 0.75	4.15 ± 1.11		2	3	
Onion powder (14)	5.40 ± 0.36	4.67 ± 0.99		3	9	2
Spray dried						
Tomato powder (8)	2.42 ± 0.43	NC[d]	7	1		
Steam treated						
Black pepper, crushed (6)	2.38 ± 0.45	NC	5	1		
Black pepper powder (7)	3.34 ± 0.53	NC	7			
Celery root powder (3)	4.20 ± 0.60	NC	2	1		
Carrot strips (10)	4.02 ± 1.33[b]	NC	6	4		
Carrot powder (3)	4.49 ± 0.44	NC	2	1		
Leek powder (3)	3.87 ± 0.70	3.25 ± 0.34		3		
Paprika granules (5)	4.39 ± 0.30	2.22 ± 0.21		5		
Paprika powder (5)	3,99 ± 0.82	NC	5			
Tomato granules (12)	4.81 ± 0.59	3.07 ± 0.59	2	10		
White pepper powder (4)	2.94 ± 0.40	2.54 ± 0.70		4		

[a] Aerobic plate count performed according to ISO 4833. Data was obtained from the spice distributor.
[b] No of samples yielding respective colony count on de Man Rogosa Sharpe (MRS) agar.
[c] Values are means ± standard deviations.
[d] Not calculated (NC) for products with most samples yielding counts below the limit of detection (2.00 log CFU/g).