International Journal of Food Microbiology 185 (2014) 7–16

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Monitoring psychrotrophic lactic acid bacteria contamination in a ready-to-eat vegetable salad production environment

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ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form 8 May 2014
Accepted 10 May 2014
Available online 15 May 2014

Keywords:
Source tracking
Vegetable salads
Psychrotrophic LAB
Leuconostoc gelidum subsp. *gasicomitatum*
Spoilage

ABSTRACT

A study monitoring lactic acid bacteria contamination was conducted in a company producing fresh, minimally processed, packaged and ready-to-eat (RTE) vegetable salads (stored at 4 °C) in order to investigate the reason for high psychrotrophic LAB levels in the products at the end of shelf-life. Initially, high microbial counts exceeding the established psychrotrophic thresholds ($>10^7$–10^8 CFU/g) and spoilage manifestations before the end of the shelf-life (7 days) occurred in products containing an assortment of sliced and diced vegetables, but within a one year period these spoilage defects became prevalent in the entire processing plant. Environmental sampling and microbiological analyses of the raw materials and final products throughout the manufacturing process highlighted the presence of high numbers of *Leuconostoc* spp. in halved and unseeded, fresh sweet bell peppers provided by the supplier. A combination of two DNA fingerprinting techniques facilitated the assessment of the species diversity of LAB present in the processing environment along with the critical point of their introduction in the production facility. Probably through air mediation and surface adhesion, mainly members of the strictly psychrotrophic species *Leuconostoc gelidum* subsp. *gasicomitatum* and *L. gelidum* subsp. *gelidum* were responsible for the cross-contamination of every vegetable handled within the plant.

1. Introduction

Technologies limiting the concentration of available oxygen (e.g. vacuum, modified atmosphere, MA) and cold storage are currently being implemented as main bacteriostatic hurdles in food packaging (Labadie, 1999; Willocx et al., 1993). This way, most notorious Gram negative, respiring, spoilage-related bacteria are inhibited and there is an increasing selection of facultative anaerobic or aerotolerant psychrotrophs. The most competitive bacterial group growing under such preservation conditions is mainly cold-acclimatized lactic acid bacteria (Borch et al., 1996; Huis in' t Veld, 1996; Pothakos et al., 2012). Although this problem concerns food products of different origins and from various production processes, it remains unclear how these LAB are introduced in production processing installations (Vihavainen et al., 2007). Air-mediated contamination is a presumptive route of introduction of spoilage-related microbiota in the food matrix, while contact with surfaces could also facilitate contamination (Audenaert et al., 2010; Johansson et al., 2011).

Members of the LAB genus *Leuconostoc* have been isolated from retail products that were deemed unfit for consumption before the end of their shelf-life (Björkroth et al., 2000; Kato et al., 2000; Lyhs et al., 2004; Sakala et al., 2002a,b; Vihavainen and Björkroth, 2007) and that showed severe spoilage manifestations (Lyhs et al., 2004; Susiluoto et al., 2003; Säde, 2011). Source tracking analyses attempting to elucidate the origin of *Leuconostoc* spp. along the food production line have displayed low recoveries (Vihavainen et al., 2007). This observation may suggest that contamination levels are initially low and thus cannot always be accurately and reliably determined by means of culture-dependent techniques. In addition, the implementation of an enrichment method for leuconostocs against a taxonomically heterogeneous microbial background (encompassing other LAB) exploiting differentiating growth traits could be undermined.

Previously, psychrotrophic LAB unable to grow at 30 °C and thus remaining undetermined during routine microbiological analysis according to the mesophilic enumeration methods (ISO 4833:2003 and ISO, 15214:1998) were found to be highly prevalent at the end of shelf-life in various samples of minimally processed, ready-to-eat (RTE) vegetable salads (Pothakos et al., 2012). *Leuconostoc* spp. were the most frequently isolated taxa exhibiting a strict psychrotrophic character and in all cases had become dominant (Pothakos et al., 2014a). The present study attempted to trace the origin of the contamination in a vegetable

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http://dx.doi.org/10.1016/j.ijfoodmicro.2014.05.009
0168-1605/

processing plant and to identify the ecological niches in which these organisms can be present.

2. Materials and methods

2.1. The processing plant and its products

This source tracking analysis was carried out in a Belgian vegetable processing plant. The company is producing fresh, minimally processed, RTE vegetable salads that are being packaged in modified atmosphere (MA) or air and that are subsequently stored at refrigeration temperature (4 °C) until the end of shelf-life (7 days). The products cover a wide range of vegetables (beetroot, broccoli, cabbage, carrots, cauliflower, celeriac, celery, corn, cucumber, endive, leek, lettuce, onion, parsley, radish, soy germs, sweet bell peppers, zucchini etc.) and are commercially packaged as single-vegetable or assortment salads. All end-products are sealed in biaxially oriented polypropylene (BOPP) film bags with 35 micron thickness and an O_2 permeability of 1400 cm^3/m^2 day. The majority of salads are filled with air except for strongly respiring vegetables (i.e. celery, mixed leafy green salad, iceberg lettuce, cucumber, radish) that are packaged under MA (5–10 % O_2: 90–95 % N_2).

For the purpose of this study, the processing plant was divided in distinct domains (Fig. 1 and Table 1) related to areas exclusively allocated to the handling of specific materials or corresponding to certain practices independent of the material (dicing, mixing, loading, packaging etc.). Domains 1 and 2 correspond to the cold storage rooms where all the raw materials and sometimes intermediate products were kept until processed or assembled with other vegetables, respectively. Domain 3 was exclusively used for coarse and fine chopping as well as washing of leafy green vegetables, whereas Domain 4 was destined for chopping and washing leek. In the latter two domains, three garbage bins were always positioned in proximity for disposing of lettuce cores and leek taproots. Domain 5 was strictly limited to an automatic rotating fine chopper for parsley. Moreover, Domains 6 and 7 occupied an area where different types of vegetables were handled through a 10 mm and 6 mm dicer, respectively. Apart from the actual machines, palettes, a balance, loading baskets and hand scoops were also in use at this specific part of the plant. Domain 8 comprised three acid baths (i.e. ascorbic acid, lactic acid, acetic acid) for dipping cabbage, cauliflower, broccoli and beetroot in order to avoid browning and a manual centrifuge. Lastly, Domains 9, 10, 11 and 12 corresponded to the four packaging lines consisting of a mixing basket, an ascending conveyor belt and the packaging/sealing machine.

2.2. Sampling

Air samples were taken to evaluate a possible air-mediated contamination, equipment and surfaces were swabbed, water samples from washing baths and tanks, raw materials, intermediate products after each handling and packaged end-products were also taken aseptically from all production lines. Sampling started at 3:30 AM ahead of the production, right after the completion of the decontamination of the plant and ended at 14:00 PM. Surface, air, vegetable and water samples were taken from each domain of the plant (Table 1) repeatedly during 10 hours.

2.2.1. Sampling of surfaces

For the surfaces and the equipment, sterile rayon swabs with plastic shaft in individual tubes (Cultiplast, Code: 111598 rayon) were used. Peptone physiological solution (PPS: 0.85 % NaCl w/v and 0.1 % peptone w/v in distilled water) was added in the tube to moisten the head of the swab. Approximately 25 cm^2 was swabbed each time and the rayon head along with the plastic shaft was directly transferred aseptically in de Man-Rogosa-Sharpe broth for assessing LAB growth, supplemented with sorbic acid (MRS-S) in order to inhibit the growth of yeasts. Samples were incubated anaerobically at 4 °C for 20 days to select for psychrotrophic LAB. The MRS-S broth was prepared from its individual components (yeast extract: 4 g/L; Lab-Lemco powder: 8 g/L; peptone: 10 g/L; sorbitan mono-oleate (Tween 80): 1 ml/L; dipotassium hydrogen phosphate: 2 g/L; sodium acetate: 5 g/L; triammonium citrate: 2 g/L; magnesium sulfate: 2 g/L; manganese sulfate: 0.05 g/L) with the addition of 1 % sorbic acid, pH was adjusted to 5.7 and autoclaved separately from the glucose (20 g/L) avoiding Maillard reactions that would alter the final pH. After autoclaving, the glucose was added in the rest of MRS-S broth and distributed in sterile glass tubes.

2.2.2. Sampling of air

The air contamination was evaluated by using a Spin air-Air sampler (IUL instruments, Spain) set at an airflow of 100 L/min and a rotation speed of 3 rpm during 8 min resulting in the sampling of 800 L air. The 90 mm petri plates used for air analysis containing MRS-S agar. After each sampling, the rotating petri plate holder and its cover were cleaned with ethanol. All plates were incubated anaerobically, at 22 °C for 5 days.

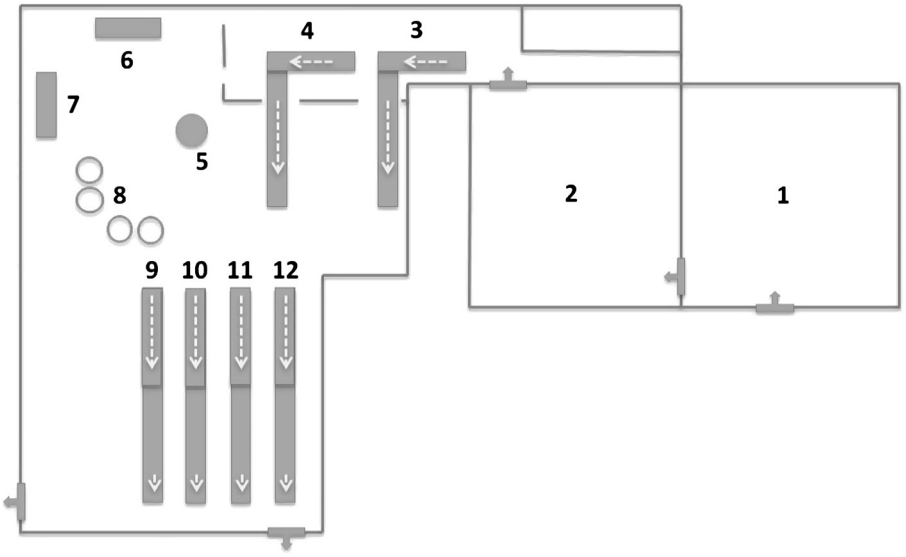

Fig. 1. Plan of the salad processing area separated in twelve domains (detailed description of each domain of the plant is presented in Table 1).