International Journal of Food Microbiology 160 (2013) 344–352

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

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

Microbiological investigation of *Raphanus sativus* L. grown hydroponically in nutrient solutions contaminated with spoilage and pathogenic bacteria

Luca Settanni [a,*], Alessandro Miceli [b], Nicola Francesca [a], Margherita Cruciata [a], Giancarlo Moschetti [a]

[a] DEMETRA Department, University of Palermo, Viale delle Scienze 4, 90128 Palermo, Italy
[b] SAGA Department, University of Palermo, Viale delle Scienze 4, 90128 Palermo, Italy

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form 30 September 2012
Accepted 3 November 2012
Available online 20 November 2012

Keywords:
Hygienic safety
Microbial transfer
Mineral nutrient solution
Raphanus sativus L.
Soilless cultivation
Undesired bacteria

ABSTRACT

The survival of eight undesired (spoilage/pathogenic) food related bacteria (*Citrobacter freundii* PSS60, *Enterobacter* spp. PSS11, *Escherichia coli* PSS2, *Klebsiella oxytoca* PSS82, *Serratia grimesii* PSS72, *Pseudomonas putida* PSS21, *Stenotrophomonas maltophilia* PSS52 and *Listeria monocytogenes* ATCC 19114^T) was investigated in mineral nutrient solution (MNS) during the crop cycle of radishes (*Raphanus sativus* L.) cultivated in hydroponics in a greenhouse. MNSs were microbiologically analyzed weekly by plate count. The evolution of the pure cultures was also evaluated in sterile MNS in test tubes. The inoculated trials contained an initial total mesophilic count (TMC) ranging between 6.69 and 7.78 Log CFU/mL, while non-sterile and sterile control trials showed levels of 4.39 and 0.97 Log CFU/mL, respectively. In general, all inoculated trials showed similar levels of TMC in MNS during the experimentation, even though the levels of the inoculated bacteria decreased. The presence of the inoculums was ascertained by randomly amplified polymorphic DNA (RAPD) analysis applied on the isolates collected at 7-day intervals. At harvest, MNSs were also analyzed by denaturing gradient gel electrophoresis (DGGE). The last analysis, except *P. putida* PSS21 in the corresponding trial, did not detect the other bacteria, but confirmed that pseudomonads were present in un-inoculated MNSs. Despite the high counts detected (6.44 and 7.24 CFU/g), only *C. freundii* PSS60, *Enterobacter* spp. PSS11 and *K. oxytoca* PSS82 were detected in radishes in a living form, suggesting their internalization.

1. Introduction

Consumers are becoming more and more shrewd concerning alimentation; thus, their attention to the damages of unhealthy foods has determined an increase in the request for vegetable products in the last years. However, in many developed countries the consumption of vegetables is lower than the recommended dietary guidelines and an increase in the consumption of vegetables and fruits is strongly advised. Nevertheless, often consumers' dissatisfaction with produce quality (appearance, flavour, texture, perishability and hygienic safety) may limit their consumption (O'Beirne, 2007).

Minimally processed vegetables are creating an increase of requests for fresh produce due to their convenience of use and attractive appearance and flavour, but their benefits are offset by the rapid deterioration/short shelf-life of the products in the marketplace and the potential health hazards associated with microbial growth. Although it is somehow difficult to determine the exact route of contamination, the environment and the agricultural practices from planting through harvesting are of particular concern (Buck et al., 2003). At the pre-harvest stage, the crop management might limit or eliminate the risks of pathogenic microbial transference from soil to vegetables (Settanni et al., 2012).

Many factors may determine the microbial contamination also during the post-harvest manipulation (Martínez-Sánchez et al., 2006); the higher the concentrations of microorganisms the more intense the washing treatment of vegetables before consumption in the fresh-cut form needs to be. For vegetables whose edible parts grow underground, soil plays a defining role on the hygienic characteristics of the products. Soil may host several microorganisms (van Elsas et al., 2007), including bacterial species that are pathogenic for humans, such as members of the Enterobacteriaceae family (Forssten, 2009), *Listeria monocytogenes* (Welshimer and Donker-Voet, 1971), or *Stenotrophomonas maltophilia* (Bollet et al., 1995) which has emerged as an important nosocomial pathogen, especially in debilitated and immunocompromised persons (Denton and Kerr, 1998). Some species of soil origin, e.g. *Pseudomonas putida* (Magnuson et al., 1990), can be responsible for the spoilage of vegetable products during storage. The pathogenic species can be transferred to humans during vegetable consumption and some fresh-cut products have been linked to food-borne outbreaks (Sivapalasingam et al., 2004; DeWaal and Bhuiya, 2007).

In order to increase yield and quality, alternative agricultural systems like soilless cultivation systems (or hydroponics) consisting of growing plants in water containing mineral nutrients (nutrient

* Corresponding author. Tel.: +39 091 23896043; fax: +39 091 6515531.
E-mail address: luca.settanni@unipa.it (L. Settanni).

0168-1605/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2012.11.011

solution) have been tested for many vegetables. Soil may be replaced by mineral or organic materials that support the roots while nutrients are diffused through the nutrient solution. In these conditions, the vegetables are cleaner and have higher sanitary quality than those from traditional (in soil) cultivation systems (Orozco et al., 2008; Selma et al., 2012) as there is no contact between soil and produce. Among soilless cultivation techniques, the floating system is a cheap and easy culture system where plants are held on panels that float on the nutrient solution.

The radish (*Raphanus sativus* L.) is a member of the Brassicaceae family grown and consumed throughout the world and its request is on the increase (Salerno et al., 2005). The edible part of this vegetable is the swollen root (derived from hypocotyl and upper radicle tissues) that can be harvested within four to six weeks from planting. Radishes are usually eaten raw for their crisp texture and pungent, peppery flavour and are also appreciated for its content of ascorbic acid, phenolic acids, anthocyanins and glucosinolates that can have a positive effect on consumer's health (Giusti and Wrostald, 1996; Lu et al., 2008; Jing et al., 2012).

Enterobacteria and *Pseudomonas* spp. have been identified within the total microbial population of radish sprouts grown in soil (Skowronek et al., 1998), confirming that radishes, if not properly treated before eating, may contribute to the spread of undesired bacteria. Islam et al. (2004) stated that a one-time application of contaminated (*Salmonella*) irrigation water or compost can result in the contamination of radishes and carrots well beyond their growing cycle.

The cultivation on floating panels is easy to apply to radishes and allows their production in an almost ready-to-eat form, since substrate and/or pesticide residues are absent and a single washing treatment could be enough before eating. Nevertheless, mineral nutrient solution, as irrigation water in soil, might play an important role in the pathogenic contamination of the plants. Hence, the objectives of the present study were: to investigate the survival of five species of Enterobacteriaceae family, *L. monocytogenes*, *S. maltophilia* and *P. putida* in mineral nutrient solution during the whole soilless crop cycle of the radish; to monitor their transference to the hypocotyl; to evaluate their viability on the radish surface at harvest; and to examine their internalization in the plants.

2. Materials and methods

2.1. Plant material and microbial strains

Radish (*R. sativus* L.) seeds cultivar Saxa 2 were purchased from Euroselect (Bari, Italy). Sterilized sand was used as a medium to germinate seeds and grow plantlets till full cotyledon development. The sand was moistened with deionized water and placed in a 4-cm layer in a glass tray. Radish seeds were placed on the surface, covered with a 0.5-cm layer of sand, and kept in the dark at 20 °C until cotyledon emergence.

Citrobacter freundii PSS60, *Enterobacter* spp. PSS11, *Escherichia coli* PSS2, *Klebsiella oxytoca* PSS82, *Serratia grimesii* PSS72, *P. putida* PSS21 and *S. maltophilia* PSS52 of food origin (Todaro et al., 2011), belonging to the culture collection of the Agricultural Microbiology laboratory of DEMETRA Department — University of Palermo (Palermo, Italy), were propagated in Nutrient Broth (NB) (Difco Laboratories, Detroit, MI). Except *P. putida* PSS21, incubated at 20 °C for 24 h, the other cultures were incubated at 37 °C for 24 h. *L. monocytogenes* ATCC 19114^T was propagated in Brain Heart Infusion (BHI) (Oxoid, Basingstoke, England) at 37 °C for 24 h.

2.2. Experimental plan

Ten trials were followed in this work (Fig. 1). Mineral nutrient solution (MNS), prepared using ground water treated with inverse osmosis (electrical conductivity 430 μS/cm; pH 7.7), contained NO_3^- (20.00 mmol/L), NH_4^+ (1.25 mmol/L), $H_2PO_4^-$ (2.00 mmol/L), K^+ (11.00 mmol/L), Ca^{2+} (5.75 mmol/L), Mg^{2+} (1.00 mmol/L), SO_4^{2-} (2.30 mmol/L), Fe^{3+} (18 μmol/L), Mn^{2+} (9 μmol/L), BO_3^{3-} (9 μmol/L), Zn^{2+} (3.8 μmol/L) and MoO_4^{2-} (3.6 μmol/L). The electrical conductivity (EC) of the MNS was 2.5 mS/cm and the pH was 6.0. Fresh microbial cultures were prepared as follows: centrifuged at 10.000×*g* for 5 min, washed in Ringer's solution (Sigma-Aldrich, Milan, Italy) and re-suspended in the same solution till reaching an optical density (OD) of ca. 1.00, measured by 6400 Spectrophotometer (Jenway Ltd., Felsted Dunmow, UK) at 600 nm wavelength, which approximately corresponds to a concentration of 10^9 CFU/mL. Plastic hydroponic boxes (13.5 cm long×9.5 cm wide×6.0 cm deep), each designed to hold 6 plants in the holes of the lid (about 468 plants/m^2), were sterilized by autoclaving at 121 °C for 20 min before filling with 650 mL of non-sterilized MNS. Cell suspensions (Cf, *C. freundii* PSS60; En, *Enterobacter* spp. PSS11; Ec, *E. coli* PSS2; Ko, *K. oxytoca* PSS82; Sg, *Ser. grimesii* PSS72; Pp, *P. putida* PSS21; Sm, *S. maltophilia* PSS52; Lm, *L. monocytogenes* ATCC 19114^T) were added singly till a final concentration of approximately 10^6 CFU/mL; a vigorous mixing was performed by means of a sterile pipette and the boxes were closed with a plastic cover with six holes. Two control conditions were included in the system: Ctrl, non-sterilized MNS non-inoculated; and sterilized Ctrl (SCtrl), filter (0.20-μm pore size filter, Sartorius, AubagneCedex, France) sterilized MNS non-inoculated. Two boxes were prepared for each trial and two independent experiments were performed in two consecutive days.

Ten days after sowing (end of March 2012), the radish plantlets with fully developed cotyledons were picked up and before immersion in MNS, sand was removed from the roots by washing with sterile water. Plants were then plugged into the holes of the lid and tightly fastened with non-absorbent cotton, so that the roots were below the cover and the hypocotyls and the cotyledons were above the cover. All 20 boxes of each repetition were moved to an unheated plastic greenhouse (SAGA Department — University of Palermo, Italy), where the radish plants were grown during spring 2012. The MNS was monitored daily for water consumption and weekly for EC and pH. Hydroponic boxes were replenished with new MNS when the volume of MNS dropped below 500 mL.

In order to evaluate the behavior in MNS without the influence of other variables, the eight pure cultures, after washing, were also inoculated (at the final concentration reported above) in sterile test tubes containing filter sterilized MNS and kept in the greenhouse for the entire radish growth cycle.

2.3. Sample collection

The sampling plan included both MNS and plants:

a) MNS (1 mL) was sampled, in duplicate for each trial, at the time of cell suspension addition (T_0) and at 7-day intervals, after thorough agitation with a sterile pipette. Each sample was collected aseptically with a portable Bunsen and a sterile pipette and transferred into a sterile test tube;
b) Plants were collected at harvest with sterile dissecting scissors and tweezers used to transfer the hypocotyls into sterile plastic bags. Two hypocotyls (one from each box) were collected from each trial for direct microbial count and two other hypocotyls were collected for the internal detection of bacteria.

The pure cultures were transferred to a sterile hood and, soon after collection (1 mL), brought back to the greenhouse.

2.4. Microbiological analysis

Microbiological analyses were performed after decimal serial dilution of pure cultures, MNS and radish samples in Ringer's solution. Radishes (approximately 10 g) were homogenized in a stomacher (BagMixer® 400, Interscience, Saint Nom, France) for 2 min at the