

Evaluation of an ELISA Method to Detect *Listeria monocytogenes* in Fresh-Cut Rocket

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Keywords: contamination, microbial, vegetables, safety

Abstract

Leafy vegetables are consumed fresh after harvest with bland washes, not always efficient in removing pathogenic bacteria that may be attached to the external skin or surfaces. In this work, an optimized ELISA based method developed in our laboratory was used to detect the presence of *Listeria monocytogenes* in fresh-cut rocket leaves. From a certain quantity (5 g) of fresh rocket leaves contaminated with *Listeria monocytogenes*, bacteria were isolated and the pathogen was detected using an ELISA protocol. The preliminary results are promising in the use of antibody-antigen interaction to verify the presence of *L. monocytogenes* in the minimally processed vegetable distribution chain.

INTRODUCTION

Ready-to-eat (RTE) vegetables are minimally processed produce that are used for a direct consumption and do not undergo further washing or cooking. Ready-to-eat vegetables have a high potential risk of foodborne contaminations since most of them are processed in several ways from in-field packing to pre-cut and bagged product. Minimally processed leafy vegetables are usually grown in soil under plastic tunnel. The baby leaf production requires frequent soil preparation and the organic matter especially during summer rapidly declines. It means that manure or other organic substances have to be distributed in order to maintain the organic matter comprised between 1.5-3%. By coming in contact with manure, leafy vegetables can be contaminated with pathogenic bacteria. The use of sewage irrigation water before harvest as well as inadequate or poor handling practices during preparation, processing and packing represent additional sources of microbial contamination (Cooley et al., 2006). Despite being washed with sanitizing agents, minimally processed leafy vegetables are consumed fresh and raw without thermal treatment and the risk of foodborne outbreak can occur. The UE regulation n. 2073/05 reports the safety criteria for different foods including ready-to-eat vegetables.

Among the human pathogenic bacteria, *L. monocytogenes* is an ubiquitous foodborne pathogen. It is a gram positive bacteria and the genus includes six species, but only the *L. monocytogenes* is pathogenic for the human. Therefore, it may contaminate a wide range of food commodities, especially fresh vegetables. *L. monocytogenes* can proliferate under antimicrobial conditions, i.e., low temperature, low water activity or low pH (Kovacevic et al., 2013). The growth potential of pathogens has been studied in nine ready-to-eat vegetables and the *L. monocytogenes* showed the highest values in wild rocket (Sant'Ana et al., 2012).

The identification of new reliable detection methods is always needed. Different methods have been tested to detect *L. monocytogenes* in different matrix, including immunoenzymatic assay, molecular biology approaches targeting both DNA or RNA and classical approaches based on selective enrichment and plating (Gasanov et al., 2005). The ready-to-eat leafy vegetables after preparation remain on the shelf of the market a period of time ranging from 3 to 5 days. In this food line, the detection methods should be very fast with the day-after result. Therefore, the aim of this work was to evaluate an ELISA assay that was optimized in our laboratory to reduce the detection time of *L. monocytogenes* in wild rocket fresh cut leaves. It does not want to substitute the current

techniques but to improve and guarantee the safety for the consumers.

MATERIAL AND METHODS

Plant Materials

Rocket (*Diplotaxis tenuifolia* L.) was purchased from supermarkets in Foggia. Overnight bacterial culture was serially 10-fold diluted and the dilutions were used to artificially contaminate rocket leaves. Spiking was realized by spraying cell suspension directly on the leaf tissue under class 2 biohazard laminary flow cabinet, allowing to dry for 15 min under sterile air flow. Rocket samples of 5 g were homogenized in 10 ml of 1× sterile phosphate buffered saline (1×PBS: 1 mM KH₂PO₄, 3 mM Na₂HPO₄, 154 mM NaCl, pH 7.2) under a laminar flow hood. Homogenized samples were incubated for 7 h at 30°C with agitation and centrifuged at 2000 rpm for 20 min at room temperature. The pellet was suspended in 2 ml of 1× PBS.

Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

Primary antibody anti-*Listeria monocytogenes* (LZA2) (ab11439) and HRP-conjugated secondary antibody (ab47827) were purchased from Abcam (Cambridge, UK) and used at concentration of 1 µg/ml and 400 ng/ml respectively. 100 µl of the isolated *L. monocytogenes* bacteria were loaded overnight at 4°C in the wells of an ELISA plate. After a four times washing step with 300 µl of PBS-T (1× PBS, 0.05% Tween20), wells were blocked with 100 µl of 1% BSA PBS-T for maximum 2 h at 25°C. After washing 100 µl of primary antibody were incubated for 1 h followed by washing and incubation with 100 µl of HRP-conjugated secondary antibody. After washing, 100 µl of TMB solution were added for 45 min at 25°C in the dark and the reaction was stopped with an equal volume of 1 N HCl or 2 N H₂SO₄; the optical density (OD) was read at 450 nm. An OD_{450nm}-fold change ≥ 2 respect to a negative control (only PBS 1× buffer) was set as threshold to distinguish positive from negative samples.

RESULTS AND DISCUSSION

Detection of *Listeria monocytogenes*

Rocket leaves were inoculated with different concentrations of *L. monocytogenes* from 10⁰ to 10⁵ cells/g. The presence of this bacteria was verified with an indirect ELISA assay (Fig. 1). The antibody recognizes the outer membrane fraction and the whole cells of *L. monocytogenes*. A total of 6 samples were tested and all of them resulted positive for the presence of *L. monocytogenes* showing an OD_{450 nm}-fold change ≥ 2 (OD-fold= OD_{450 nm} sample/OD_{450 nm} negative control). The negative control showed an OD_{450 nm} of 0.05, while the contaminated samples had a value ranging from 0.14 to 0.2 (Fig. 2). With respect to the 4-5 days long microbiological methods that require a pathogens enrichment of at least 48 h (Welshimer, 1981), the results obtained from this ELISA-based method were accomplished in 24-28 h. Other interesting molecular techniques are those that use specific primers for mRNA or DNA of the *L. monocytogenes*. The advantage of this method that is sensitive and can be also very fast reducing the detection time to few hours. On the contrary often a specific extraction and isolation methods have to be developed for the different tissues. Moreover, the primers can also amplify the nucleic acid of dead bacteria with no dangerous effect on the consumer. In literature there is also information related to other ELISA methods that have been found to detect bacteria with threshold of $\geq 10^5$ cells/ml with a time comprised from 40-48 h (Noah et al., 1991).

CONCLUSIONS

These preliminary results on rocket are promising in the use of specific antibodies to verify the presence of bacteria in a wide range of the minimally processed vegetables during the whole distribution chain.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n. 289719 (Project QUAFFETY).

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Figures

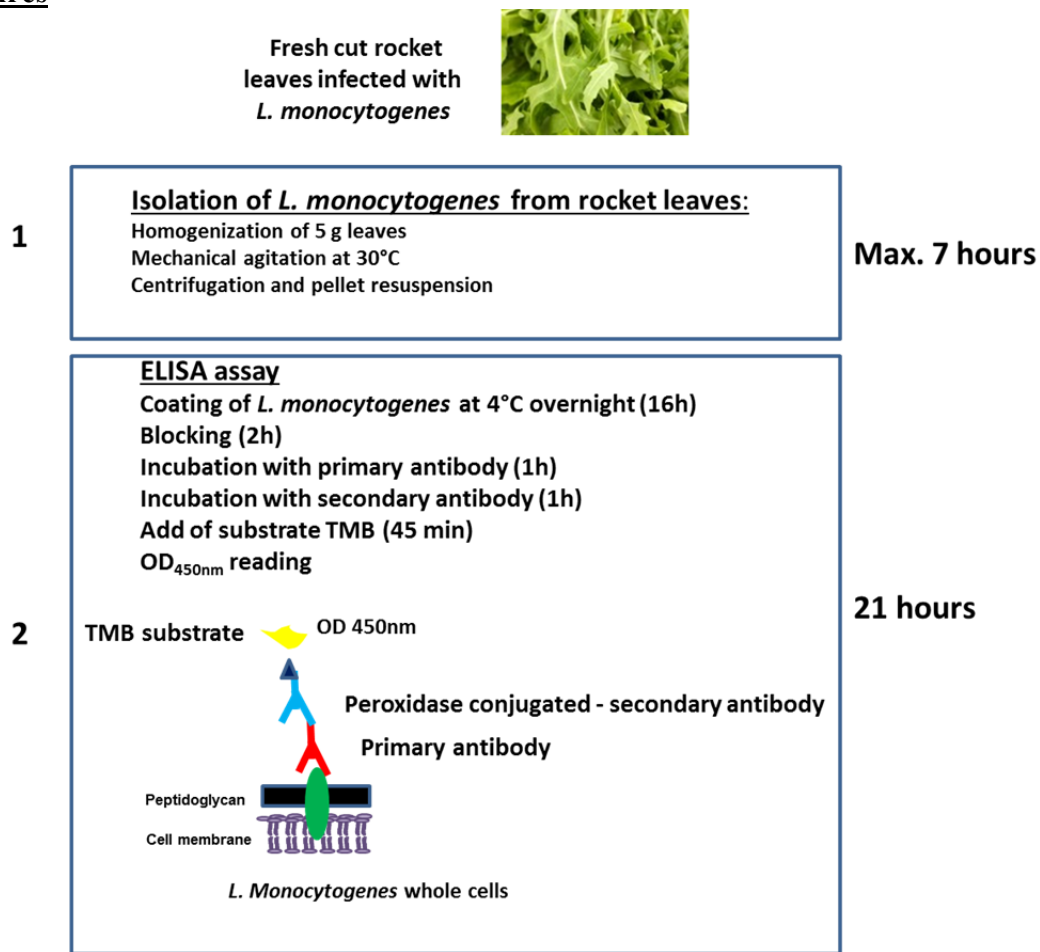


Fig. 1. Scheme of the ELISA protocol to detect *L. monocytogenes* in rocket leaves.

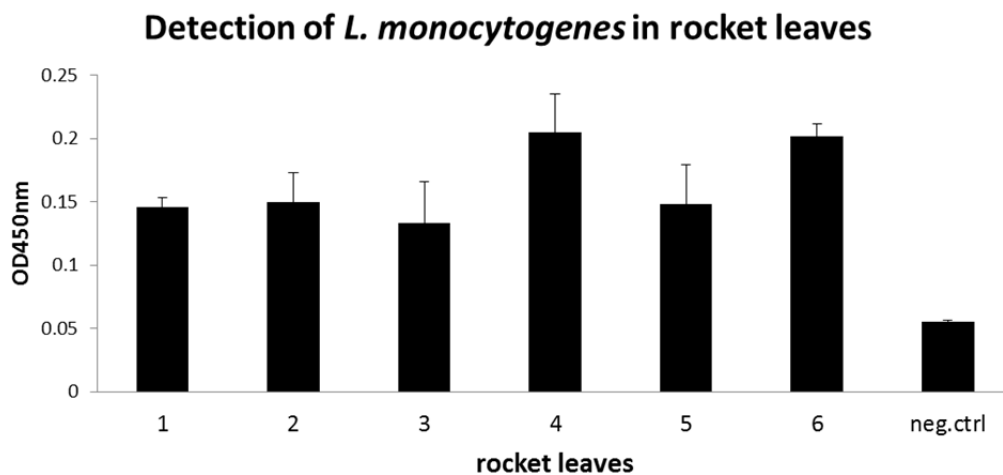


Fig. 2. Histograms showing samples versus OD_{450 nm} values. The value that showed a clear increment from the background was used to calculate the cut-off according to the following formula (Bioreba-ELISA Data analysis): mean of background values + 3SD of background values + 10%.