

A COMPARATIVE ANALYSIS OF IMMUNOASSAYS IN DETECTION OF FOOD TOXINS

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Article Received on
25 May 2018,

Revised on 15 June 2018,
Accepted on 06 July 2018

DOI: 10.20959/wjpr201814-12806

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ABSTRACT

The study focuses on comparing different methods for detecting toxins in spoiled food products. The technique of enzyme linked immunosorbent assay was used for the detection of the enterotoxins in the food samples. Different methods were applied for the immunoassay that includes Direct, Indirect and Sandwich ELISA. Our results generally demonstrated high bacterial diversity across the ten fruit, vegetables and other eatable materials. We analyzed. Eight phylogenetically diverse phyla of bacteria (*P.aeruginosa*, *B.cereus*, *S.aureus*, *S.typhii*, *Lactobacillus*, *E.coli*, *Shigella*, *L.monocytogenes*) and their toxic proteins were targeted (exotoxin A, BDE, nuc gene, invA, SlpA, STEC, stx1c, p60). Our results generally demonstrated high bacterial

diversity across the ten fruit, vegetables and other eatable materials. In the given sample of the bacteria *Listeria monocytogenes* the maximum concentration was observed for sample 10 at 3.4150 pg/ml (culture dependent) and 2.9566 pg/ml (culture Independent) for p60 protein. ELISA has become by far the most commonly employed method for in vitro detection of the toxin. Both results (culture dependent and independent) toxin detection showed not a very considerable difference.

KEYWORDS: ELISA, toxin, imuunoassay, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, culture dependent.

INTRODUCTION

Effective detection of toxins is a big challenge when a great diversity of the samples is present. Different chemical and physicochemical characteristics of the analytes lead to specific extraction, sample cleanup and detection. Therefore the detection methods are

specific for the individual toxins or for the group of similar toxins. The analysis of the toxins must be simple and should avoid the cost. Potable biosensors can be applied for the faster acquisition of the results, which enables rapid detection outside the laboratory (Hodnik and Anderluh, 2009). Fast screening of the samples is commonly performed through the enzyme linked immunosorbent assay (ELISA). The major advantage of the immunoassay is that the detection completes in less time and no special preparation of the sample is required. ELISA possess a greater selectivity and sensitivity, is easy to perform and it offers the options of simultaneous detection of various samples. Different toxic molecules are detected via different types of immunoassay techniques (Schneider *et al.*, 2004; Goryacheva *et al.*, 2007).

Immunoassays can be linked with other methods like in botulinum toxin detection. Phillips and Abbott recently reported the use of an antibody-based assay similar to an ELISA but utilizing electro-chemiluminiscent technology as an alternative to the mouse bioassay for testing food samples (Phillips and Abbott, 2008).

MATERIALS AND METHODOLOGY

Collection of spoiled Food samples

The spoiled food samples were collected from the spoiled part of food in solid and semi solid forms. The collected samples were diluted with phosphate buffer saline (PBS) (0.05 M, pH 7.4), by mixing 1 gm of spoiled food samples in 10 ml of PBS. They were thoroughly mixed then centrifuged at 10,000 rpm for 15 min and supernatant was collected and it was sterilized by filtration through a 0.22- μ m filter. These spoiled food filtrates were then assayed further.

Enterotoxins extraction from samples

The amount of food sent for enterotoxin tests varied from 0.5 g to more than 100 g. For the enterotoxin extraction the whole sample, but not more than 100 g, was extracted by the method of **Reiser, Conaway & Bergdoll (1974)**. For the enterotoxins extraction the food sample was homogenized with an equal amount of sterile distilled water for slurry formation. The extract was concentrated using polyethylene glycol (PEG). The pH of slurry was adjusted at 4-5 with 1 N HCl and then slurry was centrifuged for 10 min at 10000 rpm. After centrifugation the solid fat layer was discarded and the supernatant fluid was removed. The residue was diluted with half the amount of distilled water and originally used for centrifugation. The collected supernatant fluids were adjusted at pH 7 with 5N NaOH. If a precipitate formed, the extract was centrifuged again. The supernatant fluid was shaken with

200 of its volume of chloroform and centrifuged at 3000 rpm for 10 min. The aqueous layer was used in the ELISA both directly and after concentration in PEG.

Enzyme-linked immunosorbent assays (ELISA) for enterotoxin detection

The technique of enzyme linked immune-sorbent assay was used for the detection of the enterotoxins in the food samples. Different methods were applied for the immunoassay.

Method 1: The desired number of anti-SET antibody coated removable wells was secured and wells were allotted for each food sample, negative control and positive control. Wells were filled with wash solution and allowed to stand at room temperature for 10 minutes then removed by inverting the plate. 200 µl of the aliquots of sample and control were transferred into wells and the plate was covered with plate sealer and incubated for 2 h at 35-37°C. Then the content was removed by inverting the plate and then each well was washed with wash solution and repeated washing for 2-3 times. Each well was then loaded with 200 µl of the reconstituted enzyme and incubated at room temperature for 1 h. Then plate was washed for 5 times and 200 µl of the reconstituted substrate was added and left for 30 minutes at RT until the positive control reaches the darker color than panel no. 4 on color comparator. Then the wells were loaded with 20 µl of stop solution and result was determined via microtiter tray reader.

Method 2: The indirect ELISA was performed by coating the plate with spoiled food filtrate which contains toxin B and incubated at 4 °C for overnight. The plate was then washed and then loaded with 1% BSA solution in PBS and incubated for 1 h at 37 °C, then washed again. Plates were then loaded with a 1 in 1000 dilution of rabbit anti-toxin B in PBS containing BSA 0.2% (PBS-O.2% BSA) and incubated for 1 h at 37°C. The plates were washed again and a 1 in 2000 dilution of horseradish peroxidase conjugated affinity chromatography purified goat anti-rabbit IgG in PBS-O.2% BSA was added. After incubation again for 1 h at 37°C, the plates were washed and substrate solution was added.

Method 3: Sandwich ELISA was performed by coating the wells with sheep anti-toxin B diluted (1 in 200) dilution in sodium carbonate buffer and then incubated at 4 °C for overnight. The coated plates were usually used immediately but could be stored at 4°C for up to 4 weeks without decrease in reactivity. Uncoated attachment sites on the plates were saturated by incubation for 1 h at 37°C with BSA 1% in PBS. The plates were then washed and a solution of toxin B in PBS, alone or in the presence of control or culture medium was

added and incubated for 1 h at 37°C. The plates were washed again and incubated with the second antibody preparation (1 in 1000) dilution of rabbit antitoxin B in PBS-O.2% BSA and incubated for 1 h at 37°C and washed before the addition of (1 in 2000) dilution of peroxidase-labelled goat anti-rabbit IgG in PBS-O.2% BSA. After incubation for 1 h at 37°C, the plates were washed again and the substrate solution was added.

RESULT AND DISCUSSION

Enzyme Linked Immunosorbent assay was performed the analysis of the presence of different types of toxins present in different types of spoiled food samples. The ELISA was performed for the analysis of selective toxins produced by a specific microorganism like p60 by *Listeria monocytogenes*, stx1c by *Shigella dysenteriae* etc. The concentration of various toxins in spoiled food samples was calculated on the basis of their optical density in pg/ml. The concentration of toxins was calculated for the bacterial culture samples isolated from the spoiled food as well as from the direct extract of the food samples. The observed highest and the lowest concentration of different toxins in specific food samples is listed in the table below.

Table 1: Result of concentration (pg/ml) of various toxins in cultured samples of microbial species isolated from food samples.

Type of toxin	Culture	Sample	High conc. (pg/ml)	Sample	Low conc. (pg/ml)
P60	<i>Listeria monocytogenes</i>	Raw milk	3.4150	Mango	2.1704
Stx1c	<i>Shigella dysenteriae</i>	Bread	2.2813	Pineapple	2.0697
STEC	<i>E. coli sp.</i>	Potato	2.2180	Grapes	2.0106
SlpA	<i>Lactobacillus sp.</i>	Potato	2.2180	Raw milk	2.0464
invA	<i>Salmonella typhi</i>	Mango	2.4962	Potato	2.1920
nuc gene	<i>Staphylococcus aureus</i>	Mango	2.608108	Pear	2.263908
BDE gene	<i>Bacillus cereus</i>	Pear	2.26475	Potato	2.23495
Exotoxin A	<i>Pseudomonas aeruginosa</i>	Pineapple	2.584856	Watermelon	2.212856

Table 2: Result of concentration (pg/ml) of various toxins in direct extract of microbial food samples.

Type of toxin	Culture	Sample	High conc. (pg/ml)	Sample	Low conc. (pg/ml)
P60	<i>Listeria monocytogenes</i>	Raw milk	2.9566	Potato	2.1566
Stx1c	<i>Shigella dysenteriae</i>	Grapes	2.2707	Apples	2.1599
STEC	<i>E. coli sp.</i>	Watermelon	2.229776	Meat	2.010576
SlpA	<i>Lactobacillus sp.</i>	Bread	2.3414	Meat	2.0450
invA	<i>Salmonella typhi</i>	Pineapple	2.5292	Potato	2.1978
nuc gene	<i>Staphylococcus aureus</i>	Mango	2.659308	Raw milk	2.249708
BDE gene	<i>Bacillus cereus</i>	Raw milk	2.27015	Potato	2.23295
Exotoxin A	<i>Pseudomonas aeruginosa</i>	Pineapple	2.402656	Pear	2.207456

ELISA has become by far the most commonly employed method for in vitro detection of the toxin. For immunodetection, which is based on antigen-antibody binding selection, several types of antibodies can be used; conventional and heavy chain antibodies, as well as polyclonal, monoclonal or recombinant antibodies. For instance, detection of *L. monocytogenes* can be performed via polyclonal antibodies (Feldsine, Lienau, Forgey, & Calhoon, 1997; Jung, Frank, & Brackett, 2003) and via monoclonal antibodies (Mattingly *et al.*, 1988), but for Salmonella detection, monoclonal antibodies (Schneid, Ludtke, Diel, & Aleixo, 2005) have been used. Polyclonal antibodies have low cost and can be prepared quickly compared to its alternatives but it has low specificity and abundance (Leonard *et al.*, 2003). Monoclonal antibodies, on the other hand, have been found to be more specific and thus used in an extended range of foodborne pathogens such as *L. monocytogenes*, *Salmonella spp.*, *S. aureus*, *E. coli O157*, and *Shigella*. But it has some disadvantages too, requiring skilled workers, specialized specimen and high cost. Immunological detection methods have been studied on various techniques such as enzyme immunoassay (EIA) (Borck, Stryhn, Ersboll, & Pedersen, 2002), enzyme-linked immunosorbent assay (ELISA) (Bennett, 2005), immunochromatography (ICG) strip test (Shim *et al.*, 2007), immunomagnetic separation (Hudson, Lake, Savill, Scholes, & McCormick, 2001) etc.

Our results generally demonstrated high bacterial diversity across the ten fruit, vegetables and other eatable materials. We analyzed. Eight phylogenetically diverse phyla of bacteria (*P.aeruginosa*, *B.cereus*, *S.aureus*, *S.typhii*, *Lactobacillus*, *E.coli*, *Shigella*, *L.monocytogenes*) and their toxic proteins were targeted (exotoxin A, BDE, nuc gene, invA, SlpA, STEC, stx1c, p60). The bacterial taxa we observed were consistent with findings from other studies that have used culture-independent techniques to describe taxon abundances. A variation was observed with Ottesen *et al.* (2007) who observed that Alphaproteobacteria was the most abundant bacterial class on apples, and we found the family Enterobacteriaceae most abundant within the these samples. It is more difficult to directly compare our results with the large body of research on produce-associated bacteria that has been conducted using culture-based techniques as our study includes both culture dependent and independent detection. Furthermore, culture-based studies detect a different fraction of the bacterial community assessed using culture-independent techniques, and, in most cases, a small fraction of the total bacterial diversity (Yashiro *et al.*, 2011). In this study, we observed distinct bacterial communities and considerable variability in bacterial richness across the different samples. The protein detected from samples extracted by two different methods i.e. direct toxin

detection from food samples as well as culture bacterial toxin extraction and subsequent detection by ELISA. No considerable difference was observed in readings of ELISA in two samples.

CONCLUSION

In this study, we observed distinct bacterial communities and considerable variability in bacterial richness across the different samples. The protein detected from samples extracted by two different methods i.e. direct toxin detection from food samples as well as culture bacterial toxin extraction and subsequent detection by ELISA. No considerable difference was observed in readings of ELISA in two samples. Our results generally demonstrated high bacterial diversity across the ten fruit, vegetables and other eatable materials. In the given sample of the bacteria *Listeria monocytogenes* the maximum concentration was observed for sample 10 at 3.4150 pg/ml (culture dependent) and 2.9566 pg/ml (culture Independent) for p60 protein. In the study performed it was observed that bacteria *Shigella dysenteriae* (stx1c) showed the maximum concentration at 2.2813 pg/ml (culture dependent) for sample 9 and 2.2657 pg/ml (culture Independent). It was estimated that bacteria *E.coli* (STEC) showed the maximum concentration at 2.2180 pg/ml (culture dependent) for sample 8 and 2.135976 pg/ml (culture Independent). In the given sample of the bacteria *Lactobacillus* species (slpA) the maximum concentration was observed at 2.2180 pg/ml (culture dependent) for sample 8 and 2.0512 pg/ml (culture Independent). In the given sample of the bacteria *Salmonella typhi* (invA) the maximum concentration was observed at 2.4962 pg/ml (culture dependent) for sample 4 and 2.4806 pg/ml (culture Independent). In the given sample of the bacteria *S.aureus* (nuc protein) species the maximum concentration was observed at 2.608108 pg/ml (culture dependent) for sample 4 and 2.659308 pg/ml (culture Independent). In the given sample of the bacteria *B.cereus* (BDE) the maximum concentration was observed at 2.26475pg/ml (culture dependent) for sample 6 and 2.24155 pg/ml (culture Independent). In the given sample of the bacteria *P.aeruginosa* (exotoxin A) the maximum concentration was observed at 2.584856pg/ml (culture dependent) for sample 2 and 2.402656 pg/ml (culture Independent). We analyzed eight phylogenetically diverse phyla of bacteria (*P.aeruginosa*, *B.cereus*, *S.aureus*, *S.typhii*, *Lactobacillus*, *E.coli*, *Shigella*, *L.monocytogenes*) and their toxic proteins were targeted (exotoxin A, BDE, nuc gene, invA, SlpA, STEC, stx1c, p60). ELISA has become by far the most commonly employed method for in vitro detection of the toxin. Both results (culture dependent and independent) toxin detection showed not a very considerable difference.

ACKNOWLEDGMENT

I am highly indebted to my advisor **Dr. Vandana Pathak (Professor, Department of Physical Science, MGCGV, Chitrakoot)** for her guidance and constant supervision as well as for providing necessary information regarding the study. I express a heartfelt thanks to Mahatma Gandhi Chitrakoot Gramoday Vishwavidyalaya (Chitrakoot) for providing all the necessary facilities and support to complete this work.

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