MethodsX 4 (2017) 335–345

Contents lists available at ScienceDirect

MethodsX

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

Method Article

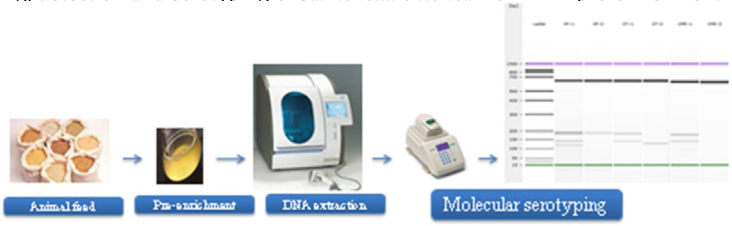
Evaluation of a multiplex PCR method to serotype *Salmonella* in animal feeds pre-enrichment broth cultures

Junia Jean-Gilles Beaubrun[a,*], Laura Ewing[a], Kim Dudley[a], Faiza Benhamed[b], Hua Wang[c], Darcy E. Hanes[a]

[a] U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Laurel, MD 207081, United States
[b] U.S. Food and Drug Administration, Center for Veterinary Medicine, Laurel, MD 20708, United States
[c] U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD 20740, United States

GRAPHICAL ABSTRACT

Screening, detection and serotyping of *Salmonella enterica* from 24 h pre-enrichment of animal feed.



Screening, detection and serotyping of *Salmonella enterica* from 24 hr pre-enrichment of animal feed.

ABSTRACT

The identification of *Salmonella enterica* serotypes remains a highly important public health concern for microbiological analysis of foods, feeds, and clinical samples. Outbreaks of human salmonellosis are sometimes linked to contact with infected animals and animal feeds. To possibly reduce the number of outbreaks, it is important to rapidly, efficiently detect *Salmonella enterica* in animal feeds and food products. A multiplex PCR for molecular serotyping of *Salmonella enterica* previously used in a single lab validation study for serotyping in multiple human food matrices was used in this investigation to evaluate the effectiveness of the multiplex PCR assay as serotyping method and screening tool for *Salmonella* in animal feeds. This approach is unique in that:

* Corresponding author at: MOD 1 Facility, Virulence Mechanisms Branch, (HFS-025), Division of Virulence Assessment, Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 8301 Muirkirk Rd., Laurel, Maryland 20708, United States.
E-mail address: junia.jean-gillesbeaubrun@fda.hhs.gov (J. Jean-Gilles Beaubrun).

http://dx.doi.org/10.1016/j.mex.2017.09.003
2215-0161/ Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

- The multiplex PCR serotyping assay may be used for rapid screening and serotyping of *Salmonella enterica* from contaminated animal feed at the non-selective pre-enrichment step.
- The assay may provide the serotype or identification of *Salmonella* in positive samples at concentration as low as 10 CFU/25 g after a 24 h non-selective pre-enrichment step.
- In addition to the ability to serotype, this assay contains *inv*A as an internal control for *Salmonella* positive identification. The *inv*A shows positive indication for *Salmonella* outside of the 30 serotypic banding patterns.

Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

ARTICLE INFO
Method name: A molecular serotyping approach for *Salmonella* enterica detection in animal feed
Keywords: *Salmonella* spp, molecular serotyping, animal feed
Article history: Received 19 February 2016; Received in revised form 29 July 2017; Accepted 8 September 2017; Available online 20 September 2017

Methods

Salmonella *identification in animal feed*

Using a method described by Benhamed et al. [5] six feeds: Wheat Brand (WB), Horse Feed (HF), Dried Molasses (DM), Calf Milk Replacer (CMR), Dried Beet Pulp (DBP), and Whole Oats (OT) obtained from commercial sources were spiked with *S. enterica* serovar Typhimurium at concentrations of 10 CFU, 50 CFU, 100 CFU per 25 g, to evaluate the detection level using a modified version of the Bacteriological Analytical Manual (BAM), Chapter 5 [1]. Each sample was pre-enriched in Lactose broth (LB) and modified buffer peptone water (mBPW) media (n = 6 replicates for each medium). Two different media were compared to determine which media was more effective and if a specific media would be more efficient for the molecular assay [11].

Then HF, WB and CMR were spiked with 10 CFU/25 g and 2.5 CFU/25 g of *Salmonella enterica* serovars Typhimurium, Agona, and Hadar, respectively to evaluate the sensitivity of the molecular assay below the microbiological assay detection level of 10CFU/25 g. Each sample was pre-enriched in Lactose broth (LB) and modified buffer peptone water (mBPW) media (n = 20 replicates for each medium). The samples were prepared as described above and then aged for 2 weeks at 4 °C. A total of 92 samples were analyzed per feed type, 40 feed samples pre-enriched in lactose broth and 40 feed samples pre-enriched in mBPW. The 12 remaining samples were the positive controls of each serovar grown in each enrichment broth and un-inoculated enrichment broths were used as negative controls. The 24 h pre-enrichment broth cultures were then transferred into selective enrichment broths and selective plating followed by serological and biochemical confirmation, using a modified version of the BAM. Subsequently, recovered colonies were identified as *Salmonella* with Vitek® 2 Compact, Version 5, (Biomerieux, St Louis, MO) [10]. Fig. 1 is a graphical demonstration of the methodology and workflow for *Salmonella* detection in animal feed.

Major equipment and supplies for PCR assay

- Vitek® 2 Compact, Version 5, (Biomerieux, St Louis, MO)
- Roche MagNA Pure Compact (Roche, Indianapolis IN)
- BioRad conventional C1000 thermocycler (BioRad, Hercules, CA)
- Eppendorf Centrifuge 5424R (Eppendorf, Hauppauge, NY)
- Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany)
- DNA 1000 Reagents kit for DNA analysis (Agilent Technologies, Waldbronn, Germany)
- PCR tubes (BioRad, Hercules, CA))
- Sterile Eppendorf style microcentrifuge tubes ((Life Sciences, Hercules, CA or equivalent)
- Sterile inoculating loops or needles (Life Sciences, Hercules, CA or equivalent)
- Ice bucket or bench top cooler
- Adjustable Micropipettors (0.1–1000 μl)

Download English Version:

https://daneshyari.com/en/article/5518482

Download Persian Version:

https://daneshyari.com/article/5518482

Daneshyari.com