A novel molecular method for the on-site detection of Salmonella in soybean meal using a POC detection device

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1. Introduction

Salmonella enterica is considered the main microbiological hazard in animal feed. Evidences collected in these years point soy and soy derivatives as the primary source of contamination of feedstock.

EFSA reports a positivity for *Salmonella enterica* of 1.5% in oilseed flours and 0.5 and 0.7% in compound feed. Hence, it is fundamental for feed industry to have a convenient method for detecting *Salmonella* in soy flours on-site preventing its diffusion in the production plant. Currently, DNA based methods are considered the most rapid in detecting the presence of *Salmonella* and other pathogens, but their use is often restricted to equipped laboratories due to complex experimental setups.

In this study, we evaluated the possibility to detect *Salmonella* in soybean meal using a crude DNA extraction buffer, a molecular detection Kit Assay for *Salmonella* spp. and a portable detection instrument driven through a mobile connection device.

2. Materials

Salmonella enrichment

- Buffered Peptone Water (BPW)
- Enrichment bag with filter
- Incubator

DNA extraction

- FASTfood Universal DNA Extraction Kit
- Dry Heat Block for 1.5 ml tubes
- 2 ml microtubes

DNA detection

- PATHfinder Salmonella spp. Detection Kit
- Hyris bCUBE 2.0, detection system

3. Sample preparation

2 samples were prepared by adding 225 mL of BPW to 25 grams of soybean meal in a stomacher bag, according to ISO 6579-1. One sample was spiked with 1 ml containing 5-10 CFU of *Salmonella enterica* serovar *Enteritidis* (ATCC 13076/WDCM 00030). Both samples were incubated 18 hours at 37°C. The concentration of *Salmonella* in the bags after incubation (Fig.1) was calculated plating 10-fold serial dilutions (using BPW as dilution buffer) of the two enriched broths onto XLD selective agar and incubated 24 ± 3 hours at 37°C ± 1°C. The measured concentration of *Salmonella* in the spiked sample was 10^8 CFU/ml while no *Salmonella* was detected in the non-spiked sample. While waiting for the plate counting the enriched broths were stored at 4°C.

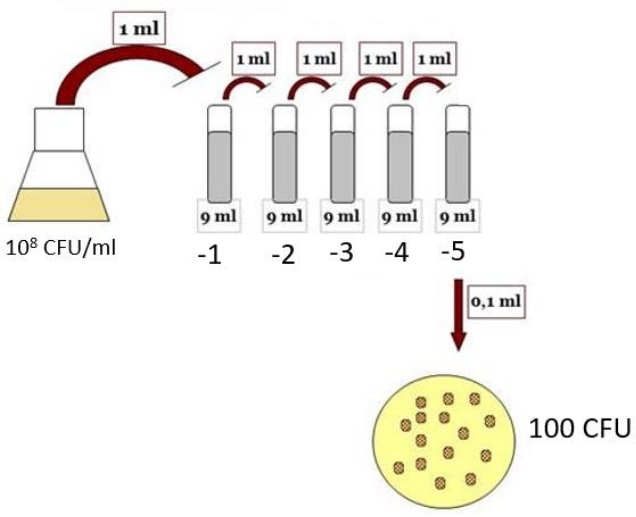

Figure 1 **Plating of 10-fold serial diluted samples onto XLD selective plates**: After 18 ± 2 hours of BPW pre-enrichment, 10-fold serial dilutions were made from both presumed negative and contaminated samples (from -1 to -5). For each dilution 0,1 ml was plated onto XLD selective agar and after 24 ± 3 hours of incubation, 100 CFU were counted on the last one. Therefore, it can be assumed that the initial concentration of the enriched starting spike sample was approximately 10^8 CFU/ml.

4. Molecular detection

The PATHfinder Salmonella spp. kit was previously demonstrated to have a LOD<10 genomic copies of *Salmonella* per reaction; however, to define the experimental LOD it is paramount to evaluate the minimal concentration in CFU/ml *Salmonella* must reach in the broth to be eventually detected by the molecular reaction. Thus, multiple samples with scalar concentration of *Salmonella* and equal amounts of bystander flora were created sequentially diluting 10-fold the spiked broth with the non-spiked enriched broth.

Bacterial DNA of each of these samples was extracted mixing 100 µl of broth with 100 µl of FASTfood Universal DNA Extraction Buffer in a microtube and incubating 15' at 98°C on a dry heat-block. Afterwards, microtubes were spin at 3000 rpm for 1 minute to precipitate debris. *Salmonella* DNA was detected by adding 5 µL of clear DNA extract to 15 µL of PATHfinder Salmonella spp. supermix (prepared as specified in product insert) and running the amplification reaction on a Hyris bCUBE system using a 36 wells cartridge. The DNA extract of the sample containing 10^8 CFU/ml showed positivity after few amplification cycles (Fig. 2) while no *Salmonella* DNA was detected in the extract derived from the broth of the non-spiked sample.

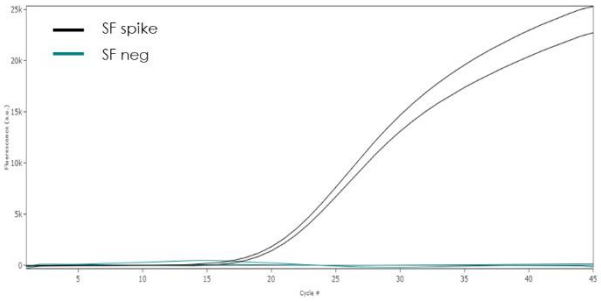

Figure 2 **Data exported from Hyris bAPP platform**: This data was obtained using the following "Defatted" Soy Flour (SF) samples: Negative (neg) with FAST FOOD sample prep and 5-10 CFU spike prior to enrichment (spike) with FAST FOOD sample prep.

Salmonella DNA can be reliably detected in the extracts originated from the diluted samples down to the fourth decimal dilution (Fig. 3) hence demonstrating that Salmonella shall reach a concentration of 10^4 CFU/ml in the enrichment broth to be detected in the molecular reaction. This is consistent with other molecular method present in the market or documented in scientific literature.

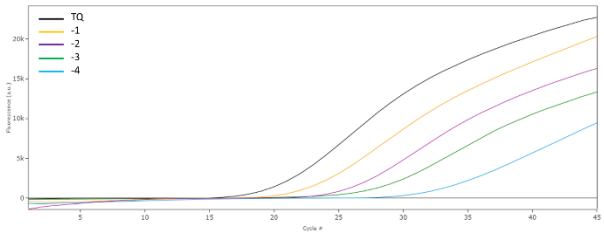

Figure 3 **Limit of Detection (LOD) Study**: Data exported from Hyris bAPP platform. Samples consisted of negative enriched "Defatted" Soy Flour spiked with known amounts: 10^8 (TQ), 10^7 (-1), 10^6 (-2), 10^5 (-3), 10^4 (-4) of Salmonella Enteritidis ATCC 13076/WDCM 00030 and prepared using the FASTfood sample prep.

5. Conclusion

This study suggests that *Salmonella* can be detected in contaminated soybean meal in less than 24 hours using a convenient method based on Salmonella DNA detection. We showed the capacity to detect Salmonella when the enrichment does not reach completion. This is of peculiar importance because Salmonella present in soybean meal might be stressed by chemical treatments thus limiting its replication capacity. An appropriate method verification is mandatory when implementing the protocol in a production facility possibly using naturally contaminated (incurred) samples.

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