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Novel electrochemical identification and semi quantification of bovine constituents in feedstuffs

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Abstract

Identifying contaminating bovine constituents in feed has been a major means to help prevent the spread of bovine spongiform encephalopathy (BSE). The phenomenon of DNA aggregation induced by Hoechst 33258 in conjunction with the change in anodic current measurement was, for the first time, applied for bovine DNA detection in feedstuffs. By using the PCR amplification system specific to bovine parathyroid and common 12S rRNA genes, anodic current peaks measurements of these PCR products on linear sweep voltammetry could be determined. Anodic peaks measurement of bovine parathyroid gene among ruminant meat and pet foods containing bovine constituents were at 1.18–1.52 μA while anodic peaks among non bovine samples were greater than 1.78 μA. In the study, anodic current peaks greater than 1.75 μA could be used to distinguish non-bovine from other samples in a qualitative analysis. For quantitative analysis, bovine content was measured using the comparative ratio between copy number of bovine parathyroid and 12S rRNA genes. This ratio reflected the proportion of target bovine cells to total cell numbers. In the experiment, contents of bovine constituents in four kinds of tested pet foods were 10.88, 8.76, 6.39 and 2.69%. Compared with the first two samples on which defined content had been addressed, the estimated content with 90.66 and 87.60% accuracy could be obtained, respectively. Although, this quantitative detection was not a real-time determination, the method had several merits on its rapidity and simplicity in performing the test and on its cost effectiveness since no sophisticated devices and expensive reagents were needed.

Keywords: Electrochemical DNA sensor; Electrochemical identification; Hoechst 33258; BSE; Bovine DNA detection; Pth gene; Feedstuffs

1. Introduction

Enforcing the regulation on meat blood and bone meal in feed for farm animal production especially ruminants is considered an important measure to prevent unsafe foods due to the risk of bovine spongiform encephalopathy [1].

For this specific purpose, a microscopic examination of animal constituents in animal feeds is the only official method available [2]. However, this method requires skillful and experienced staff and cannot identify the animal species involved. The alternative application of immunological methods was similarly less advantageous since the identification of heat-treated components contained in feedstuffs was practically difficult due to thermal protein denaturation [3]. DNA based methods have been demonstrated to be a reliable tool for food and feed analysis [4] with advantages such as a degree of specificity and applicability even in thermally processed products.

So far, several DNA methods based on PCR in combination with restriction enzymes (PCR–RFLP) or PCR alone (PCR/RAPD) have been developed [5,6]. Result analysis of those methods, however, requires further steps of analysis on restriction endonuclease or sequence or banding profiles, which are time consuming and provide only qualitative data.

Recently, a biosensor detection system using electrochemistry has been demonstrated for nucleic acid based diagnosis [7]. For detection, the system needs a biomolecule that has a highly specific recognition element and a transducer that converts a molecular recognition event into a quantifiable signal. A demonstration of signal transduction using electrochemicals had been carried out using a DNA probe, a modified gold electrode, and Hoechst 33258 as a label [8]. However, all of those methods required laborious steps of immobilizing the

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probe DNA on the surface of the electrode before hybridization.

In a previous report, our group had developed a method based on electrochemical biosensor that required no probe and immobilization steps [9]. The method employed the aggregation phenomenon of the target DNA in the presence of Hoechst 33258. The aggregation of the DNA provides a clue to quantifying DNA at a trace level through the measurement of anodic current peaks. Anodic current decreases in proportion to the titration of dsDNA aggregations. Success in the fabrication process of the electrode unit, which can be integrated into the system for detection of DNA in reaction solution without DNA purification, enables the detection of target DNA in a rapid, less intensive, and cost effective fashion.

In this report, we applied DNA aggregation induction by Hoechst 33258 to the bovine DNA detection in feedstuffs by integrating the capability and specificity of the PCR technique for a qualitative analysis. We then measured the amplification ratio between a known single DNA copy of bovine parathyroid gene (Pth) and the common eukaryotic 12S ribosomal RNA (rRNA) gene to find a relationship between both. The content of bovine constituents was calculated based on number of bovine related cells detected via Pth gene per total cells detected via 12S rRNA gene. This is the first DNA detection system that demonstrates the application of DNA binding ability of Hoechst 33258 molecules to target DNA materials, and the application of the electrochemical biosensor principle in the detection of bovine DNA species in feedstuffs.

2. Experimental procedures

Meats, feedstuffs and DNAs (Table 1) were extracted from samples obtained from commercial sources using 300 mg samples as starting materials. Confirmation of the meat species was according to Wolf et al. [10]. Design of primers was based on the bovine Pth gene (K01938) and a 12S rRNA gene [8,21]. Selected primers, (5′-tataaaagtcacattgaagggtctacag-3′ and 5′-tgtaagaaagaactcatggaaacttaaa-3′ for the Pth gene and

Table 1
Detail descriptions of samples tested, and the comparative results between qualitative detection of bovine specific Pth DNA through anodic current peaks measurement and results from gel electrophoresis

Sample no.	Components in label or in notification (major to minor)	Characteristic	Country of origin	Confirmation of meat species[a]	Anodic peak (μA), (SD), and judgement	DNA detected[b]	Result bovine or non-detected
1. Cloned target DNA	–	DNA solution	–	ND	0.88(0.09)/+	+	Bovine positive control
2. Soybean genome DNA	–	DNA solution	–	ND	2.06(0.08)/−	−	Non-detected negative
3. Non-template control	–	–	–	ND	2.02(0.07)/−	−	Non-detected control
4. Poultry meat	–	Sliced meat	Japan	Poultry	1.83(0.06)/−	−	Non-detected
5. Ruminant meat	–	Sliced meat	Japan	Bovine	1.18(0.06)/+	+	Bovine
6. Pork meat		Sliced meat	Japan	Porcine	1.82(0.06)/−	−	Non-detected
7. Pork meal	Porcine	Powder	Netherlands	Porcine	1.80(0.08)/−	−	Non-detected
8. Porcine protein	Porcine	Powder	Denmark	Porcine	1.76(0.05)/−	−	Non-detected
9. Porcine meal	Porcine	Powder	Denmark	Porcine	1.78(0.07)/−	−	Non-detected
10. Poultry by-product meal	Poultry	Pellet	Netherlands	Poultry	1.81(0.08)/−	−	Non-detected
11. Nutrition	Poultry	Pellet	France	Poultry	1.85(0.05)/−	−	Non-detected
12. Pet food pellet A 15% protein	Bovine	Pellet	France	Bovine	1.28(0.08)/+	+	Bovine
13. Pet food pellet B 15% protein	Bovine poultry	Pellet	Australia	Bovine and poultry	1.36(0.06)/+	+	Bovine
14. Pet food soup 8% protein	Bovine Poultry	Cube meat	Netherlands	Bovine and poultry	1.42(0.08)/+	+	Bovine
15. Pet food pellet 17.6% protein	Bovine poultry	Pellet	Japan	Poultry and bovine	1.52(0.06)/+	+	Bovine
16. Pet food slice and pellet 30% protein	Mixed species	Pellet	Australia	Poultry and porcine	1.88(0.06)/−	−	Non-detected

ND, not determined; +, detected; −, not detected.
[a] Tested according to Wolf et al. [10].
[b] Determined on agarose gel electrophoresis.