DETECTION METHOD OF CHICKEN CONTAINED IN PROCESSED FOODS BY POLYMERASE CHAIN REACTION

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Introduction

Food allergies have recently been recognized as a clinical phenomenon in developed countries with an estimated 8 % of children and 2 % of adults having some sort of food allergy (Jansen *et al*., 1994; Bousquest *et al*., 1998). To avoid consumption of processed food containing potentially allergenic ingredients, sufficient information regarding the content of such ingredients is necessary. In Japan, the Ministry of Health, Labor and Welfare has enforced a labeling system for allergenic food ingredients since April 2002; thus, labeling indicating the content of eggs, milk, wheat, buckwheat and peanuts is now mandatory and it is recommended for twenty other food ingredients.

Allergy to chicken meat is quite rare, and no case of anaphylactic shock caused by allergy to chicken meat has been reported. But chicken meat is included in the twenty food ingredients for which labeling is recommended. Therefore, a method to detect chicken meat in processed food is required. At present, there is a PCR method that allows us to distinguish chicken meat from mammalian meat (Matsunaga *et al*., 1999), but there is none to distinguish chicken meat from other types of avian meat. In this study, we attempted to differentiate chicken genomic DNA from that of other poultry by PCR amplification of the 16S ribosomal RNA region contained in chicken mitochondria.

Materials and Methods

PCR primers: A chicken specific primer pair was designed to amplify the 102-bp segment of the 16S rRNA gene, based on multiple alignment analysis (Genetyx-Win software, Software Development Co.,Tokyo, Japan) for chicken, quail, duck and turkey sequence (accession numbers NC007236, NC003408, L22479 and AY850900, respectively).

Sample selection: DNA was extracted from chicken meat (broiler chicken, Nagoya-cochin, gamecock and Hinai-jidori), avian meat (duck, quail and turkey), mammalian meat (pork, beef and mutton), fish, shellfish, and cereals. DNA was also extracted from commercially available processed foods that contained chicken meat (canned food, retort curry, French-fries and granulated soup).

Preparation of model processed foods: Pork was used as the matrix to prepare the model processed foods. Defatted freeze-dried chicken meat powder was mixed with defatted freeze-dried pork powder at 0, 100, 10, 1, 0.1 0.01, 0.001 and 0.0001 %. Distilled water was added to the mixed powder and the mixture was separated into three equal portions. One portion was boiled at 100 ºC for 10 min, another was autoclaved at 120 ºC for 15 min, and the third one was left untreated.

DNA isolation: Genomic DNA was extracted from each sample using an anion exchange-type kit (Genomic-tip 20/G, Qiagen, Hilden, Germany) according to a previous report (Watanabe *et al*., 2006).

PCR amplification: Genomic DNA was amplified by PCR using the ExTaq PCR System (Takara Bio, Kyoto, Japan). The PCR conditions were 95°C for 3 min, 95°C for 30 sec, 69° for 30 sec and 72°C for 10 min for 30 cycles, followed by incubation at 68°C for 7 min. PCR products were separated by 2 % agarose gel electrophoresis.

Results and Discussion

After PCR of DNA extracted from chicken meat (broiler chicken, Hinai-jidori , Nagoya-cochin and gamecock), quail, turkey, duck, beef, pork and mutton, only that from chicken was amplified (Figure 1).

The reaction to ingredients other than chicken meat was investigated, but no DNA amplification was obtained for chicken eggs (yolk and white), fishery products (small clam, clam, flatfish, sea bream, salmon and codfish) or grains (corn, broad beans and green peas) (Figure 2).

The method sensitivity for detecting DNA from chicken was examined using the model foods containing a trace amount of chicken. As a result, it was possible to detect as little as 0.001% (10 ppm) of chicken component in the untreated , boiled and autoclaved model foods (Figure 3).

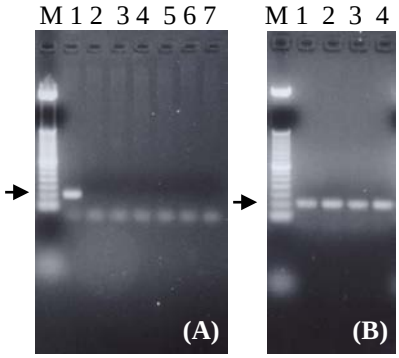

Figure 1 PCR amplifications of chicken and other edible meat.
(A) M:100bp DNA ladder marker, 1:chicken, 2:quail, 3:duck, 4:turkey, 5:beef, 6:pork, 7:mutton
(B) M:100bp DNA ladder marker, 1:broiler chicken, 2:gamecock, 3:Nagoya-cochin, 4:Hinai-jidori
The arrows show the specific detection(102bp).

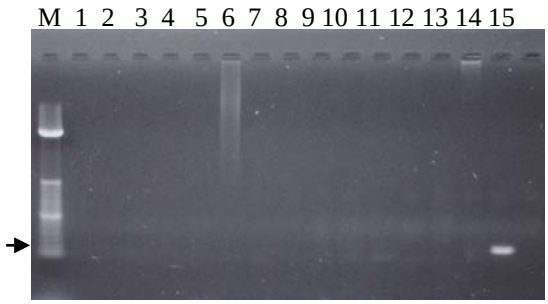

Figure 2 The PCR amplifications to ingredients other than meat.
M: 100bp DNA ladder marker, 1:small clam, 2:clam, 3:sea bream, 4:flatfish, 5:codfish, 6:salmon, 7:Kombu(kelp), 8:corn, 9:broad beans, 10:green peas, 11:egg(white), 12:egg(yolk), 13:boiledegg(white), 14:boiled egg(yolk), 15:chicken

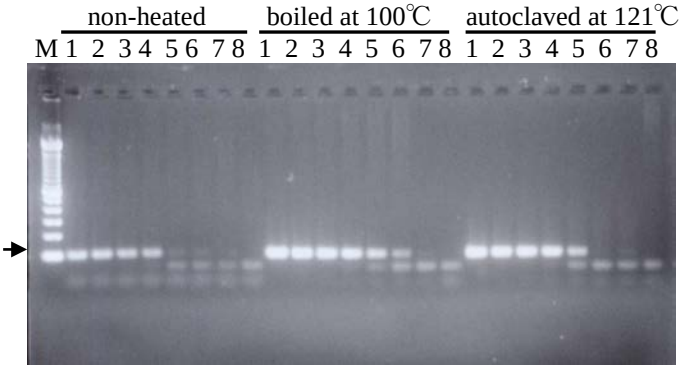

Figure 3 The method sensitivity for detecting chicken DNA from model processed foods contained in chicken meat powder.
The mixture ratios of chicken meat powder is as follows;1:100%, 2:10%, 3:1%, 4:0.1%, 5:0.01%, 6:0.001%, 7:0.0001%, 8:0%, M:100bp DNA ladder marker

Conclusion

The PCR by the primer designed in this study was specific for the DNA from chicken, and did not amplify the DNA from other birds used in the food industry (quail, duck and turkey). It was able to detect 10ppm chicken meat contained in heated foods.

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