

Construction of ELISA System to Detect NPTII Protein in Genetically Modified Foods

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Abstract

An ELISA detection system specific for neomycin phosphotransferase II (NPTII) protein was constructed using the antibodies against overexpressed NPTII protein in recombinant *Escherichia coli*. NPTII protein in genetically modified (GM) tobacco plants could be qualitatively detected using this system; however, non-specific detection in normal tobacco, soybean, corn and potato was not observed. These results suggest that the constructed ELISA system can monitor NPTII protein in GM foods.

Key words: genetically modified (GM) food, neomycin phosphotransferase II (NPTII), ELISA

I. Introduction

GM foods are being circulated throughout the world and therefore, the importance of their safety assessment was proposed. The safety assessment criteria vary among different countries. In Japan, the stability of the inserted DNA, potential toxicity and allergenicity of an expressed protein, secondary effects due to gene expression etc., have to be assessed according to the safety assessment, which is mandatory required by the Food Sanitation Law.

NPTII catalyzes the ATP-dependent phosphorylation of the 3'-hydroxyl group in the aminohexose region of certain aminoglycosides, including neomycin and kanamycin. The gene coding NPTII (*nptII*) has been used as a selectable marker to distinguish GM organisms. The *nptII* is contained in various kinds of vector plasmids and is transferred to cells with the objective gene(s). In some GM foods, that have already been approved in Japan, namely, corn, potato, sugar beat, cotton, the *nptII* has been used as a marker gene during the preparation of the recombinant. However, it is not removed and is expressed in cells after the foods are commercialized. Hence, it is necessary to evaluate the *nptII* during the safety assessment of GM foods similar to the assessment of genes that confer resistance to herbicides, insects, viruses, etc. There have been no reports regarding the adverse

effects of either NPTII or the *nptII* on humans, animals or the environment (Miki and McHugh, 2004). It has also been mentioned that the *nptII* has been appropriately evaluated, and there are no safety concerns for the time being (Food Safety Commission Decision, 2004). In future, the marker gene should be removed for consumer acceptance based on the strategies for creating marker-free transgenic plants (Miki and McHugh, 2004). On the other hand, it is necessary that the safety of the *nptII* or the NPTII protein is continuously monitored to avert unintended or unexpected effects.

In this paper, in order to monitor the stability of the NPTII protein and the expression of the *nptII*, an ELISA detection system was constructed. The antibodies for the ELISA system were prepared from the NPTII protein that was overexpressed in recombinant *E. coli*. The constructed ELISA system could qualitatively detect the NPTII protein expressed in a genetically modified tobacco plant.

II. Materials and Methods

1. Construction of NPTII expression vector

The strategy for the construction is shown in Fig. 1. The *nptII* was amplified using the primer pair of 5'-CATATGATTGAACAAGATGGATT-3' and 5'-