Qualitative analysis of *Sitophilus granarius* (L.) using real-time PCR

Furui, S.*#[1], Miyanoshita, A.[1], Imamura, T.[1], Minegishi, Y.[2], Kokutani, R.[2], Murai, T.[2]
[1]National Food Research Institute, National Agriculture and Food Research Organization (NARO), 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan
[2]Nippon gene Co., Ltd., 1-8-7, Toiya-machi, Toyama 930-0834, Japan

*Corresponding author, Email: satfurui@affrc.go.jp
#Presenting author, Email: satfurui@affrc.go.jp

DOI: 10.14455/DOA.res.2014.44

Abstract

Insects can be identified by specific morphological characteristics. Polymerase chain reaction (PCR) assay to examine differences in DNA sequences of living organisms is widely used in various identification tests. This analytical method is highly sensitive and is also applicable for the verification of hereditary diseases as well as to criminal investigations. For the identification of insects, the Cytochrome C oxidase I (COX1) gene of mitochondrial DNA (mtDNA) is often used. However, to select the best DNA region for insect identification, not only the COX1 gene but also whole mtDNA information should be used. In this study, we have developed some methods to identify *Sitophilus granarius* (L.) by real-time PCR, which compares the whole mtDNA sequence information *Sitophilus zeamais* Motschulsky, *Sitophilus oryzae* (L.), and *S. granarius*. DNA extraction and purification of total DNA containing mtDNA was performed using DNeasy® Blood & Tissue Kits (Qiagen). The whole mtDNA sequences were analyzed by a next-generation sequencer (GS Junior bench top system, Roche Diagnostics), and determined about 70% of the nucleotide sequence for each species. Some granary-weevil-specific regions were chosen to compare with 3 mtDNA information, and real-time PCR methods based on Taq-man® chemistry for identification were developed. Specificity tests conducted with several insect DNAs including 3 weevils described above, revealed that designed primer/probe sets for real-time PCR are highly specific to targets.

Keywords: weevil, detection, real-time PCR, next-generation sequencer, mitochondrial DNA