DEVELOPMENT OF A DUPLEX REAL-TEAM PCR METHOD FOR THE DETECTION OF ALL RELEVANT CRUSTACEAN SPECIES IN FOOD

Main author: Vincent Blaschke (German Federal Institute for Risk Assessment - BfR)

Co-authors: Jutta Zagon, Matthias Winkel, Thao Uyen Tran, Mohammad Naneh, Albert Braeuning

INTRODUCTION

Worldwide, approximately 3.5-4.0 % of the adult population suffer from food allergies that can cause severe problems such as an anaphylactic shock syndrome (Taylor, 2008). With over 67 000 species, the crustaceans are the biggest subphylum of the phylum arthropod (Worms, 2021). It is estimated that 1-2.5 % of the population is affected specifically by a crustacean allergy. Currently, there is no cure or treatment for food allergies, so consumers with allergies have to rely on proper food labelling as the only way to avoid allergenic foods that can cause life-threatening reactions.

METHODOLOGY

We developed a highly sensitive duplex real-time PCR method for the detection of the nuclear 18S rRNA gene of the crustacean suborder Pleocyemata and the mitochondrial 16S rRNA gene of the suborder Dendrobranchiata. Both suborders belong to the order Decapoda, which comprises about 96 % of all crustaceans consumed as human food.

RESULTS

Our duplex system uses two primer sets with the same annealing temperature at 60 °C. We were able to detect all commercially relevant crustacean species. In total, we cross-tested over 100 different species, 20 species of crustaceans and eight different food products to ensure a high specificity. The sensitivity of the PCR system was confirmed as 1 pg DNA by validation experiments. Specificity, sensitivity (LOD12 of 1 pg) and robust tests verified our system.

DISCUSSION

The high degree of diversity in the crustacean phylum makes detecting all relevant species with the same sensitivity a very complicated and difficult process. Various systems for the

detection of crustaceans have already been established (Zagon et al., 2017, Mäde-Rohmberger, 2017 and Eischeid-Stadig, 2018). The duplex real-time PCR system that was designed and developed for the detection of all commercially relevant crustaceans has been successfully implemented in this study. With this new system, we managed to circumvent problems of previous PCR systems that often contained many primer pairs from different systems, and have established a universal crustacean system that detects all commercial species used as food.