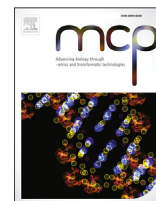
Molecular and Cellular Probes 29 (2015) 99–115

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr



In silico and experimental evaluation of DNA-based detection methods for the ability to discriminate almond from other *Prunus* spp.

Barbara Brežná [a, *], Jiří Šmíd [a, d], Joana Costa [b], Jan Radvanszky [c], Isabel Mafra [b], Tomáš Kuchta [a]

[a] Department of Microbiology and Molecular Biology, Food Research Institute, Priemyselná 4, P.O. Box 25, 824 75 Bratislava 26, Slovakia
[b] REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[c] Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 83334 Bratislava, Slovakia
[d] Institute of Food Chemistry and Biotechnolgy, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 612 00 Brno, Czech Republic

ARTICLE INFO

Article history:
Received 19 March 2014
Accepted 26 November 2014
Available online 5 December 2014

Keywords:
Almond
DNA
Real-time PCR
Food
Allergen

ABSTRACT

Ten published DNA-based analytical methods aiming at detecting material of almond (*Prunus dulcis*) were *in silico* evaluated for potential cross-reactivity with other stone fruits (*Prunus* spp.), including peach, apricot, plum, cherry, sour cherry and Sargent cherry. For most assays, the analysis of nucleotide databases suggested none or insufficient discrimination of at least some stone fruits. On the other hand, the assay targeting non-specific lipid transfer protein (Röder et al., 2011, Anal Chim Acta 685:74–83) was sufficiently discriminative, judging from nucleotide alignments. Empirical evaluation was performed for three of the published methods, one modification of a commercial kit (SureFood allergen almond) and one attempted novel method targeting thaumatin-like protein gene. Samples of leaves and kernels were used in the experiments. The empirical results were favourable for the method from Röder et al. (2011) and a modification of SureFood allergen almond kit, both showing cross-reactivity $<10^{-3}$ compared to the model almond.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

An analytical method for the detection of almond in food is necessary to protect almond-allergic consumers, as well as for authentication of food products containing almond. In this sense, DNA-based methods have demonstrated their usefulness for species identification and food allergen detection [1]. Within the last decade, the development of a reliable method for the detection of almond has been attempted by several research teams [2–14], as highlighted in the recent review by Costa et al. [15]. This interest has arisen from the increasing numbers of sensitised/allergic individuals to various food components, including almond. Food allergy affects approximately 5% of young children and 3–4% of adults in western countries [16]. The prevalence of almond allergy in Australian children was estimated to be 0.02% [17]. Data specifically regarding almond allergy in the general population are scarce, as it is often monitored in connection with allergy to tree nuts. The designation of tree nuts is considered an artificial category created for the allergological purposes, and besides almond, it comprises hazelnut, walnut, pecan nut, cashew nut, Brazil nut, pistachio nut and macadamia nut. These species are not closely related at botanical level, but in terms of allergy their cross-reactivity is frequent [16].

In sensitised individuals, the consumption of offending food may lead to various adverse effects, ranging from mild clinical symptoms (skin rash and swelling), up to severe immunological reactions such as anaphylaxis and death. So far, the safest way to cope with an allergy is the total avoidance of the offending food. For this reason, the composition of pre-packaged foods must be available for the consumers and correctly described on the respective label. As a result, almond, as part of tree nuts, is on the list of ingredients required to be labelled according to the legislation of EU [18], USA, Canada, Australia, New Zealand, Argentina, Bolivia, Chile, Colombia, Costa Rica, Cuba, Mexico, Nicaragua and Venezuela [19].

Almond (*Prunus dulcis* or *Amygdalus communis* L.) is closely related to several stone fruits of the genus *Prunus*. For the food industry, the most important related species are peach (*Prunus persica*), apricot (*Prunus armeniaca*), plum (*Prunus domestica*), cherry (*Prunus avium*) and sour cherry (*Prunus cerasus*). In some foods, almonds may be simultaneously present with some of these related species.

* Corresponding author.
E-mail addresses: brezna@vup.sk, barbarabrezna@gmail.com (B. Brežná).

http://dx.doi.org/10.1016/j.mcp.2014.11.006
0890-8508/© 2014 Elsevier Ltd. All rights reserved.

Confectionery products such as chocolates or candies, which are likely to contain undeclared traces of almond, may additionally comprise dried or otherwise processed tissues of peach, apricot, plum, cherry or sour cherry. Marzipan is a product consisting primarily of ground almonds and sugar, being prone to adulteration owing to the addition of other ingredients, such as apricot, peach or plum kernels that do not comply with the designation of marzipan [20].

Since the presence of almond in food products may also have legal consequences, a specific reliable method to differentiate almond from other genetically related plant species is of high importance for food control laboratories. Until now, the development of a specific method for unequivocal almond identification has been difficult to accomplish. The biological relatedness of *Prunus* species is very high, as mirrored by the high similarity of DNA sequences in the homologous genes, which complicates the development of discriminatory PCR methods [11]. The distinction between almond and peach is particularly difficult due to their high degree of similarity, being actually capable of forming hybrids. Almond and peach crosses are regularly used as rootstocks for almonds in the agriculture [21].

Although some published methods and commercial kits have been made available for the detection of almond, many authors admit cross-reactivity between almond and other stone fruits [6,8,9,11,13], while others never checked for cross-reactivity during the method validation [2–4,7,10]. On the other hand, the attempt of increasing the specificity of the DNA marker for almond detection may lead to the choice of a variable region as a possible target. In this case, since the target region may not be identical in all almond cultivars, a certain level of false negativity of the assay might occur. For a fair evaluation and comparison of the available methods for almond detection, validation on a broad panel of almond cultivars and other stone fruits is required. Apart from experimental testing, which provides a direct evidence of specificity of the methods, *in silico* analysis is also a valuable source of information. This approach may be of interest; in particular, if some methods were designed long time ago when less *Prunus* sequences were available in the public databases.

In this study and to the best of our knowledge, we intended to perform for the first time, the *in silico* evaluation of all published almond detection DNA-based assays regarding their potential cross-reactivity to food-relevant species of the *Prunus* genus. It was also aimed to experimentally test a critically selected subset of methods based on real-time PCR, including a commercial kit and an approach based on a novel potential target gene.

2. Materials and methods

2.1. In silico analysis

Within the target sequences of previously published methods for the detection of almond, the relevant parts covering positions of oligonucleotide hybridisation regions were found. These fragment sequences were submitted to online programme Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared to the following databases separately: nucleotide collection (nr/nt), expressed sequence tags (EST), genomic survey sequences (GSS) and whole genome shotgun contigs (WGS). The programme was limited to search only those GenBank data that complied with the following Entrez query: "*Prunus dulcis*"[Organism] OR "*Prunus persica*"[Organism] OR "*Prunus armeniaca*"[Organism] OR "*Prunus domestica*"[Organism] OR "*Prunus avium*"[Organism] OR "*Prunus cerasus*"[Organism] OR "*Prunus sargentii*"[Organism] OR "*Prunus dulcis* × *Prunus persica*" [Organism] OR "*Prunus persica* × *Prunus dulcis*" [Organism]. The resulting alignments (Fig. 1) were evaluated manually for the presence of mismatches in DNA regions of primer hybridisation from each target sequence.

2.2. Plant materials and samples

Samples of almonds and other *Prunus* species were obtained from the following sources: National Clonal Germplasm Repository, Davis, California, USA (leaves); Dr. Fernando Ponz, Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain (leaves); Botanic Garden, Slovak Agricultural University, Nitra, Slovakia (leaves); orchards located in Southwest region of Trás-os-Montes, Northeast of Portugal (kernels); Slovakian grocery stores and outdoor markets (kernels) and private gardens (leaves) (Supplemental Table 1).

2.3. DNA extraction

DNA was extracted from leaves by chaotropic solid phase extraction (SPE) using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. From kernels, DNA was extracted by chaotropic SPE using NucleoSpin Food Kit (Macherey–Nagel, Düren, Germany) according to the manufacturer's instructions.

2.4. Estimation of DNA concentration

Concentration of DNA extract from Ferraduel almond, used as model, was measured fluorimetrically in triplicate, using PicoGreen dye and bacteriophage lambda DNA as calibrator, according to the instruction attached to the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Paisley, UK). The concentration of other samples was estimated indirectly by universal real-time PCR, using decimal dilutions of Ferraduel almond DNA as calibration standard. For comparison, two alternative universal markers, the 18s rRNA and cytochrome c oxidase subunit VIa precursor genes were used (Supplemental Table 1). In case of suspected PCR inhibition (no amplification, atypical amplification curves or geometric standard deviation higher than 3), the samples were 4-fold serially diluted and reassessed.

2.5. Sequencing of SureFood kit amplicon

The SureFood® Allergen Almond real-time PCR kit (R-Biopharm, Darmstadt, Germany) was used according to the manufacturer's instructions. The product of almond-specific reaction obtained from almond DNA template of Vama cv. was purified using QiaQuick PCR purification kit (Qiagen, Hilden, Germany), cloned by QIAGEN PCR Cloning Kit (Qiagen, Hilden, Germany) and transformed into DH5alpha competent cells. Transformants were screened by PCR using plasmid-targeting primers M13F-40 and M13R, as described elsewhere [22]. The same primers were subsequently used for sequencing, which was performed at the Department of Molecular Biology, Faculty of Natural Sciences, Bratislava, Slovakia. Sequences were obtained for the cloned insert of greater size, i.e. SureFood kit amplicon, as well as for shorter insert, i.e. dimer of SureFood primers.

2.6. Design of oligonucleotides

Oligonucleotides targeting the marker of SureFood kit were designed as follows. Sequence of cloned SureFood primer dimer was compared to SureFood amplicon, and the probable sequence of the original primers was deduced. The amplicon sequence was aligned to homologous *Prunus* sequences available in NCBI databases to confirm the presence of primer mismatches towards *Prunus* other than almonds (Fig. 2). The probe was designed *de novo* by means of Primer3 software (http://frodo.wi.mit.edu/). The criteria for probe design were set to obtain a maximum of 35 nucleotide

Download English Version:

https://daneshyari.com/en/article/2199665

Download Persian Version:

https://daneshyari.com/article/2199665

Daneshyari.com