Z Ernährungswiss 36: 155–160 (1997)

ORIGINAL CONTRIBUTION

R. Greiner
U. Konietzny
K.-D. Jany

Is there any possibility of detecting the use of genetic engineering in processed foods?

Ist der Nachweis des Einsatzes der Gentechnik bei verarbeiteten Lebensmitteln möglich?

Summary To elucidate if there is any possibility to identify highly processed foods as produced through genetic engineering, beer, soya bean oil, processed tomato (ketch-up, paste, pizza tomatoes, peeled tomatoes, soup) and potato (french fries, crisps, mashed potatoes, flour, starch, fried potatoes) products as well as an enzyme preparation (Natuphos) were investigated by PCR. In pizza tomatoes, peeled tomatoes, french fries, fried potatoes, potato flour and potato crisps DNA suitable for PCR was found. Therefore, it is possible to identify these products as produced through genetic engineering. Such an identification is impossible in certain beers (pilsener, export, Nutfield lyte), soya bean oil, tomato soup, potato starch, mashed potatoes and Natuphos since PCR-analysis gave no indication of the presence of DNA in these products. As it was shown by adding *Escherichia coli* DNA the used method is, in principle, capable of detecting specifically small amounts of DNA in such products.

Zusammenfassung Bier, Sojaöl, verarbeitete Tomaten- (Ketchup, Mark, Pizzatomaten, Schältomaten, Suppe) und Kartoffelprodukte (Pommes frites, Chips, Püree, Mehl, Stärke, Bratkartoffeln) sowie ein Enzympräparat (Natuphos) wurden mittels PCR daraufhin untersucht, ob ein Nachweis des Einsatzes der Gentechnik bei ihrer Herstellung möglich ist. PCR-fähige DNA ließ sich aus Pizzatomaten, Schältomaten, Pommes frites, Bratkartoffeln, Kartoffelmehl und Kartoffelchips isolieren, so daß der Nachweis des Einsatzes der Gentechnik bei deren Herstellung möglich wird. Bestimmte Biere (Pils, Export, Nutfield lyte), Sojaöl, Tomatensuppe, Kartoffelstärke, Kartoffelpüree und Natuphos entziehen sich einem solchen Nachweis, da die PCR-Analyse keine Hinweise auf das Vorliegen von DNA in diesen Produkten ergab. Daß das durchgeführte Nachweisverfahren grundsätzlich in der Lage ist, geringe Mengen an DNA auch in diesen Produkten spezifisch nachzuweisen, wurde nach Zugabe von *Escherichia coli* DNA bestätigt.

Key words Detection method – genetic engineering – polymerase chain reaction – processed food

Schlüsselwörter Gentechnik – Nachweisverfahren – Polymerasekettenreaktion – verarbeitete Lebensmittel

Abbreviation *PCR* = polymerase chain reaction · *DNA* = desoxyribonucleic acid

Received: 13 November 1996
Accepted: 5 February 1997

R. Greiner (✉) · U. Konietzny · K.-D. Jany
Bundesforschungsanstalt für Ernährung
Engesserstraße 20
76131 Karlsruhe, Germany

Introduction

In the meantime, isolated products or processed foods deriving from genetically engineered organisms which no longer contain viable genetically engineered organisms are on the European market. In Great Britain tomato paste deriving from genetically engineered tomatoes has been sold with the corresponding labelling since spring 1996. Furthermore, clearance of soya products (oil, protein) and canola products (oil) deriving from the corresponding genetically engineered plants as food or food ingredients was announced in 1994 (1) and 1995 (2), respectively. As such products do not contain viable genetically engi-

neered organisms they are allowed to be introduced in all member states of the European Union.

Obviously, detection methods for foods produced through genetic engineering could be based on the newly introduced genetic information. Because of its high sensitivity, its specificity and rapidity the polymerase chain reaction (PCR) will be the method of choice for this purpose. The PCR is already used in the food area (8), for example, to detect and type pathogenic microorganisms in food samples (4, 12, 18) or to characterize and assure quality of food products (3, 15, 16). In the near future, a further application of the PCR may be the identification of foods deriving from genetic engineering with regard to checking the legal regulations (9). Requirements for such a detection method are the availability of adequate intact recombinant deoxyribonucleic acid in the food and the knowledge of the genetic modification (necessary to design specific PCR-primers).

Intact DNA is available, if the food is the genetically engineered organism itself, such as tomatoes and potatoes, or if the food contains genetically engineered organisms, such as yoghurt with lactic acid bacteria. Therefore, it should be no problem to develop a method to identify raw products and less processed foods as produced through genetic engineering by adapting a common DNA extraction procedure. The successful identification of genetically engineered plant products by PCR was already demonstrated by Meyer (17) using the "Flavr Savr"-tomato and by Jongedijk et al. (10) using a transgenic potato. Furthermore, it was shown by Lick et al. (13) that it is possible to isolate DNA suitable for the PCR-assay from yoghurts containing viable lactic acid bacteria and from thermally treated yoghurt, respectively. The German working group "Development of methods to identify foods produced by means of genetic engineering" which already performed interlaboratory studies on potatoes, raw sausages and yoghurt fermented with genetically modified microorganisms. The group successfully applied for the inclusion of two methods (6, 7) in the "Official Collection of Methods according to Article 35 of the German Food Act".

However, food processing is one of the main factors influencing the accessibility of appropriate nucleic acid substrate for PCR reactions negatively. During food processing fragmentation of DNA could occur, for example, by mechanical treatment (shear-forces), enzymatic (nucleases) and chemical hydrolysis (acidic pH). Moreover, food processing may lead to a complete degradation or removal of the DNA. Therefore, the possibility of an identification of a processed food as produced through genetic engineering has to be elucidated on a case-by-case basis. The aim of our work was to investigate, if there is any possibility to identify certain processed foods as produced through genetic engineering.

Materials and methods

Materials

Beer (pilsener, export), soya bean oil and processed tomato (ketchup, paste, tinned pizza tomatoes, tinned peeled tomatoes, soup) and potato (french fries, crisps, mashed potatoes, flour, starch, fried potatoes) products were purchased from local supermarkets. Tomato paste deriving from genetically engineered tomatoes was obtained from Zeneca Plant Science (Bracknell, UK), Nutfield lyte (a beer produced through genetic engineering) from BRFInternational (Redhill, UK) and Natuphos from BASF (Ludwigshafen, Germany). *Escherichia coli ATCC 33965* was obtained from E. Merck (Darmstadt, Germany). Deoxynucleotide triphosphates and Taq DNA-polymerase were products of Perkin Elmer (Weiterstadt, Germany). The following kits were used according to instructions of the supplier: DIG-nucleic acid detection kit, DIG-oligonucleotide-3-endlabelling kit (Boehringer-Mannheim, Germany) and Qiagen plasmid midi kit (Diagen, Hilden, Germany).

DNA extraction, from *E. coli* and *Saccharomyces cerevisiae* cells

Chromosomal DNA from *E. coli ATCC 33965* and *Saccharomyces cerevisiae* was isolated according to Maniatis et al. (14). Extracted DNA was concentrated by ethanol-precipitation and resuspended in TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). DNA concentration was estimated by measuring absorbance at 260 nm. *E. coli* DNA was partly digested by Sau3A and serially diluted with TE-buffer prior to addition to the food samples.

DNA extraction from food samples

Tinned pizza tomatoes, tinned peeled tomatoes, french fries, potato crisps, mashed potatoes, potato flour, potato starch, fried potatoes, Natuphos, soya beans, potatoes, tomatoes

Food samples were pulverized in a mortar under liquid nitrogen. 300 mg of the food samples were suspended in 1 ml extraction puffer (100 mM Tris-HCl, 1.0 M sodium chloride, 20 mM EDTA, 2 % (w/v) SDS, pH 8.0 containing 500 $\mu g\ ml^{-1}$ proteinase K). The samples were incubated for 60 min at 60 °C and centrifuged at 12 000 g in a microcentrifuge for 10 min. 600 µl of the aqueous phases were transferred to fresh microfuge tubes and phenol/chloroform/2-pentanol extraction was carried out.

Tomato ketchup, tomato paste, tomato soup

The food samples were lyophilized prior to pulverization under liquid nitrogen. The DNA extraction was carried out as described above.

Beer, soya bean oil

5 ml of beer or soya bean oil was thoroughly mixed with 45 ml of TE-buffer. After centrifugation the aqueous phases were loaded onto equilibrated Qiagen-tip 100 columns. The Qiagen-tip 100 columns were run according to the instructions of the supplier. For DNA precipitation, the DNA containing eluates were mixed with 0.1 volumes of 3 M sodium acetate, pH 5.0 and 2 volumes of ethanol (98 %). The samples were incubated at -18 °C for 2 h. The precipitated DNA was pelleted by centrifugation at 12 000 g in a microcentrifuge for 10 min at 4 °C, washed once with ice cold (-18 °C) 70 % ethanol, air dried and resuspended in 100 µl of water. Those samples were directly used for PCR-analysis.

Phenol/chloroform/2-pentanol extraction and RNase digestion

DNA containing samples were mixed thoroughly with the same volume of a phenol:chloroform:2-pentanol mixture (25:24:1, v/v/v). After centrifugation at 12 000 g in a microcentrifuge for 5 min, the aqueous phases were transferred to fresh microfuge tubes. The same volume of a chloroform:2-pentanol mixture (24:1, v/v) was added and mixed thoroughly. The phases were separated by centrifugation at 12 000 g in a microcentrifuge for 5 min. DNA was precipitated by adding 0.1 volumes of 3 M sodium acetate, pH 5.0 and 2 volumes of ethanol (98 %). The samples were incubated at -18 °C for 2 h. The precipitated DNA was pelleted by centrifugation at 12 000 g in a microcentrifuge for 10 min at 4 °C, washed once with ice cold (-18 °C) 70 % ethanol, air dried and resuspended in 100 µl of 0.1 M Tris-HCl, pH 7.5. RNase was added to a final concentration of 100 µg ml^{-1} and the samples were incubated for 30 min at 25 °C. Thereafter a phenol/chloroform/2-pentanol extraction and a DNA precipitation was performed. The precipitated DNA was resuspended in 100 µl of water and those DNA samples were used for PCR-analysis.

Addition of *E. coli* DNA

To all food samples 1 ml of the Sau3A digested *E. coli* DNA solution was added to give between 10 ng and 100 fg DNA per gram of product. Thereafter, the corresponding above described DNA extraction methods were performed.

Oligonucleotide primers, PCR conditions and analysis of PCR products

The sequences of the oligonucleotide primers are given in Table 1. The oligonucleotides EC1 and EC3 were previously described to detect *E. coli* DNA in model systems for foods deriving from genetic engineering (11) and the oligonucleotides TRO3 and TRO4 to identify soya bean DNA in meat products (3, 15). The oligonucleotides TMPA3 and TMPA4 were based on the DNA sequence of the polygalacturonase-encoding gene of tomatoes (20). The sequences of the oligonucleotides SOLSUC1 and SOLSUC3 were chosen on the basis of the sucrose synthase-encoding gene of potatoes (19). The oligonucleotides APHY1 and APHY2 were directed against the sequence of the acid phosphatase-encoding gene (*PHO 5*) of *Saccharomyces cerevisiae* (5) and the oligonucleotides PHYA1 and PHYA2 were deduced according to the sequence of the phytase-encoding gene (*phyA*) of *Aspergillus niger var. ficuum* (21).

Table 1 Oligonucleotides used as PCR-primers

Primer	oligonucleotide sequence (5'→3')	Tm (°C)	Annealing temperature (°C)	Predicted size of the PCR product (bp)
EC1	GCTAATCGCCTATCTCGGAC	58.0	54.0	660
EC3	CTAATAACGGGGTGGCGCGG	71.0		
SOLSUC1	GTGAGCGTGTTGATGCAACT	61.9	55.0	238
SOLSUC3	GGCCTCAAACGAATAGCAAG	62.1		
TRO3	GCCCTATCAACTTTCGATGGTA	63.8	60.0	137
TRO4	ATTTGCGCGCCTGCTGCCT	74.8		
TMPA4	GGTTATCCAAAGGAATAGTA	52.3	50.0	254
TMPA3	CACCCTTAGCACCAAAGCTA	60.9		
APHY1	GATGGTTTAGGTGACCAATT	56.1	50.0	201
APHY2	GGTCAAGTTCAAACCCTTGT	58.8		
PHYA1	CTCTCGACCCAGGCACCTGC	70.1	65.0	198
PHYA2	CGACGGTGCTGGTGGAGATG	69.5		

Amplification was performed in 0.2 ml tubes using 25 µl total reaction volumes containing 1.0 µl samples of extracted DNA. The reaction mixture consisted of 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001 % (w/v) gelatin, 0.5 µM of each primer, 200 µM of each deoxynucleotide triphosphate and 0.5 units of Taq DNA-polymerase in a Perkin-Elmer GeneAmp PCR System 2400. While using the TRO3 and TRO4 primers the reaction conditions were 37 °C for 30 min (preincubation) and then 45 cycles of 95 °C for 5 s (denaturation), 68 °C for 30 s (primer annealing) and 72 °C for 10 s (primer extension) followed by a final extension at 72 °C for 2 min (3). For all other primer pairs the following conditions were used: an initial DNA denaturation step at 95 °C for 3 min followed by 40 cycles of primer annealing at the temperatures given in Table 1 for 30 s, primer extension at 72 °C for 1 min and denaturation at 95 °C for 30 s. A final extension was carried out at 72 °C for 5 min. Amplified products were held at 4 °C until analyzed by standard agarose gel electrophoresis followed by ethidium bromide staining.

Results and discussion

Since there is only a very limited number of processed foods produced through genetic engineering available, conventionally produced processed foods were used to investigate the possibility of isolating DNA suitable for the PCR. Those foods only represent models for foods deriving from genetic engineering. Since there is no fundamental difference in production processes for certain foods by using genetic engineering, a successful amplification by PCR indicates that the food under investigation could be identified as produced through genetic engineering if primers corresponding to the newly introduced DNA sequences, were made available. Furthermore, two products produced through genetic engineering were included into the investigation; tomato paste deriving from genetically engineered tomatoes and beer produced with a genetically engineered yeast. Processing of raw materials to foods may lead to a fragmentation of the DNA. To increase the probability of obtaining amplification products while using DNA isolated from highly processed foods PCR-primers were chosen, so that rather small amplification products (137–254 bp) should be obtained (Table 1).

First, the different primer pairs were tested and optimized with DNA isolated from the corresponding pure organisms. In all cases an amplification product of the expected size was obtained. Detection limits of these PCR-analysis were determined to be 5 pg genomic tomato or potato DNA, 100 pg genomic soya bean DNA and 500 fg genomic yeast DNA. The lower sensitivity while using soya bean DNA as target DNA may be attributed to the relative high fat and protein content of soya beans, since strong inhibitory effects of mixtures of protein and fat on PCR were already described (11, 18). If no inhibitory factors interfere with the PCR, 1 to 10 target molecules are sufficient to allow successful amplification and subsequent identification (13). While using complex food matrices as a DNA source the effectiveness of the PCR may be negatively influenced by several food compounds, typically by reducing the detection limits as compared to those found using pure DNA. In some cases the PCR could even be completely stopped by certain food compounds (3, 18). It has to be kept in mind that only a very few substances will reach concentrations where they are inhibitory on their own, but individual compounds can become concentrated by the extraction method employed.

Since only low molecular mass DNA would be expected in processed food, the DNA extraction procedure was followed by a RNase digestion to differentiate between DNA and RNA. From tinned pizza tomatoes, tinned peeled tomatoes, french fries, fried potatoes, potato flour and potato crisps DNA suitable for PCR could be extracted (Table 2). With that, it was demonstrated that there is a possibility to identify the above-mentioned processed foods as produced through genetic engineering. The use of genetic engineering will be impossible to detect in certain beers (pilsener, export, Nutfield lyte), soya been oil, tomato soup, potato starch and mashed potatoes since agarose gel electrophoresis and PCR-analysis gave no indication of the presence of DNA in these products (Table 2). To investigate if the used procedure is capable of detecting specifically small amounts of DNA in such products and to determine the detection limits, *E. coli* DNA was added to the food samples before performing DNA extraction and PCR-analysis. To simulate the fragmentation of DNA during food processing the *E. coli* DNA was partly digested by Sau3A prior to addition to the food samples. The target sequence of the PCR primers (EC1, EC3) was the phytase-encoding gene of *E. coli*. The specificity of the EC1 and EC3 primers for *E. coli* was examined using purified DNA from *E. coli* and DNA isolated from the food samples without added *E. coli* DNA. Using the PCR conditions described PCR products were not observed with DNA from the food samples. The sensitivity of the PCR assay was assessed using purified *E. coli* DNA (17). The PCR assay detects 50 fg *E. coli* DNA (equivalent to approximately 10 copies of the phytase-encoding gene). It was shown that the applied detection procedures are capable of detecting low numbers of phytase-encoding genes from *E. coli* in all the food samples (Table 2). Therefore, difficulties in extracting DNA from the food samples or reduction of sensitivity of the PCR by several food compounds were not the reasons for the negative findings with certain beers, soya bean oil, tomato soup, potato starch and mashed potatoes, but these products do not contain detectable amounts of DNA. The differences in sensitivity may be due to the existence of different food matrices

Table 2 PCR-analysis of processed foods and detection limits after adding *E. coli* DNA

Food sample	PCR product	Detection limits[1]
beer	not detectable	100
soya bean oil	not detectable	100
tomato ketchup	not detectable	10 000
tomato paste	not detectable	10 000
tinned pizza tomatoes	detectable	5 000
tinned peeled tomatoes	detectable	5 000
tomato soup	not detectable	10 000
french fries	detectable	1 000
potato crisps	detectable	5 000
mashed potatoes	not detectable	5 000
potato flour (starch)	not detectable	5 000
potato flour	detectable	5 000
fried potatoes	detectable	1 000
enzyme preparation[2]	not detectable	10 000

[1] determined after adding *E. coli* DNA (gene copies per gram of food sample)
[2] Natuphos

and the application of different DNA isolation procedures, respectively (Table 2). The detection limits for the foreign gene were estimated to be between 10^4 gene copies per gram of food sample (tomato ketchup, tomato paste, tomato soup) and 10^2 gene copies per gram of food material (beer, soya bean oil).

Since enzymes are the primary gene products and because of their widespread use in food processing the far biggest progress in using genetic engineering in the food area was achieved in producing enzymes in genetically engineered microorganisms. Those enzymes are used for food processing and the corresponding foods are on the market. Therefore, a enzyme preparation (Natuphos, BASF) was investigated for the possibility to detect the use of genetic engineering in its production process. Natuphos is used as a feed additive to improve phosphate and mineral bio-availability in monogastric animals. No amplification product was observed by PCR using a DNA extract from the enzyme preparation and PCR primers (PHYA1, PHYA2) targeting the phytase-encoding gene of the production organism. The sensitivity of the PCR assay was assessed to about 10 gene copies using a pure plasmid containing the phytase-encoding gene from *Aspergillus niger var. ficuum*. After addition of *E. coli* DNA to the enzyme preparation the expected PCR product was obtained applying the same DNA extraction procedure. Detection limits were determined to be 10^4 gene copies per gram of the enzyme preparation.

This work illustrates how effective the extraction and detection methods described are for rapidly and specifically detecting of foreign DNA in food samples, since the applied DNA extraction methods are capable of providing an appropriate nucleic acid substrate for the PCR from food samples as shown after adding *E. coli* DNA. It was shown that, in principle, food derived from genetic engineering could be identified by detection of the newly introduced genetic information, but processing reduces the probability of such an identification or even prevents it. A clear identification of a processed food as produced through genetic engineering is possible in exceptional cases only, since processing is one of the main factors influencing the accessibility of appropriate nucleic acid substrate for the PCR assay negatively by degradation and removal of the DNA, respectively.

References

1. Advisory Committee on Novel Foods and processes (ACNFP) (1995) Annual Report 1994. Department of Health and Minestry of Agriculture, Fisheries and Food
2. Advisory Committee on Novel Foods and processes (ACNFP) (1996) Annual Report 1995. Department of Health and Minestry of Agriculture, Fisheries and Food
3. Allmann M, Candrian U, Höfelein Ch, Lüthy J (1993) Polymerase chain reaction (PCR): a possible alternative to immunochemical methods assuring safety and quality of food. Z Lebensm Unters Forsch 196:248–251
4. Allmann M, Höfelein Ch, Köppel E, Lüthy J, Meyer R, Niederhauser Ch, Wegmüller B, Candrian U (1995) Polymerase chain reaction (PCR) for detection of pathogenic microorganisms in bacteriological monitoring of dairy products. Res Microbiol 146:85–97
5. Bajwa W, Meyhack B, Rudolph H, Schweingruber AM, Hinnen A (1984) Structural analysis of the two tandemly repeated acid phosphatase genes in yeast. Nucl Acid Res 12:7721–7739
6. BgVV (1997) Untersuchung von Lebensmitteln: Nachweis einer gentechnischen Veränderung von Kartoffeln durch Amplifizierung der veränderten DNA-Sequenz mit Hilfe der PCR (Polymerase Chain Reaction) und Hybridisierung des PCR-Produktes mit einer DNA-Sonde. L 24.01-1. In: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedarfsgegenstände / BgVV. Loseblattausgabe, Stand Februar 1997, Berlin, Köln: Beuth Verlag GmbH
7. BgVV (1997) Untersuchungen von Lebensmitteln: Nachweis einer gentechnischen Veränderung von *Lactobacillus curvatus* in Rohwurst durch Amplifikation der veränderten DNA-Sequenz mit Hilfe der PCR (Polymerase Chain Reaction) und Hybridisierung des PCR-Produktes mit einer DNA-Sonde. L 08.00–44. In: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedarfsgegenstände/BgVV. Loseblattausgabe Februar 1997, Berlin, Köln: Benth Verlag GmbH
8. Candrian U (1994) Die Polymerase-Kettenreaktion in der Lebensmittelanalytik. Mitt Gebiete Lebensm Hyg 85:704–718
9. Greiner R (1996) Analytische Möglichkeiten der Erkennung gentechnisch veränderter Lebensmittel. Getreide, Mehl und Brot – accepted for publication

10. Jongedijk E, de Sutter AAJM, Stolte T, Van den Elzen PAM, Cornelissen BJC (1992) Increased resistance to potato virus X and preservation of cultivar properties in transgenic potato under field conditions. Bio/Technology 10: 422–429
11. Konietzny U, Greiner R (1996) Model systems to develop detection methods for food deriving from genetic engineering. J Food Comp Anal – accepted for publication
12. Kwaga J, Iverson JO, Misra V (1992) Detection of pathogenic *Yersinia enterocolitica* by polymerase chain reaction and Digoxigenin-labelled polynucleotide probes. J Clin Microbiol 30: 2668–2673
13. Lick S, Keller M, Bockelmann W, Heller KJ (1996) Optimized DNA extraction method for starter cultures from yoghurt. Milchwissenschaften 51:183–186
14. Maniatis T, Fritsch EF, Sambrook J (1989) Molecular cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, Cold Spring Harbour
15. Meyer R, Candrian U, Lüthy J (1993) Tierartenbestimmung und Sojanachweis in erhitzten Fleischprodukten mittels Polymerase-Kettenreaktion (PCR). Mitt Gebiete Lebensm Hyg 84:112–121
16. Meyer R, Candrian U, Lüthy J (1994) Detection of pork in heated meat products by the polymerase chain reaction. J Assoc Off Anal Chem 77:617–622
17. Meyer R, (1995) Nachweis gentechnologisch veränderter Pflanzen mittels der Polymerase Kettenreaktion (PCR) am Beispiel der FLAVR SAVR™-Tomate. Z Lebensm Unters Forsch 201:583–586
18. Rossen L, Nørskov P, Holmstrøm K, Rasmussen OF (1992) Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. Int J Food Microbiol 17:37–45
19. Salanoubat M, Belliard G (1987) Molecular cloning and sequencing of sucrose synthase cDNA from potato (*Solanum tuberosum* L.) preliminary characterization of sucrose synthase mRNA distribution. Gene 60:47–56
20. Sheehy RE, Pearson J, Brady CJ, Hiatt WR (1987) Molecular characterization of tomato fruit polygalacturonase. Mol Gen Genet 208:30–36
21. van Hartingsveld W, van Zeijl CMJ, Harteveld GM, Gruka RJ, Suykerbuyk MEG, Luiten RGM, van Paridon PA, Selten GCM, Veenska RE, van Gorcom RFM, van den Hondel CAMJJ (1993) Cloning, characterization and overexpression of the phytase-encoding gene *(phyA) of Aspergillus niger.* Gene 127:87–94