Chapter 12

Surface Plasmon Resonance Genosensor for the Detection of *Fusarium culmorum*

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Abstract

Surface plasmon resonance (SPR)-based DNA biosensors have been shown to be rapid, label-free, and selective tools for the detection of PCR products. Here, we describe an SPR sensor based on DNA hybridization for the detection of *Fusarium culmorum*, a fungal pathogen of wheat. A 0.57 kb DNA fragment of *F. culmorum* was amplified by specific primers, and a 25-mer oligonucleotide probe was selected within the sequence of the PCR amplicon. The biotin-labeled probe was immobilized on a streptavidin sensor chip and tested for biospecific interaction with PCR products of *F. culmorum*. The SPR biosensor was applied to the detection of *F. culmorum* in fungal cultures and in naturally infected wheat samples.

Key words: DNA biosensor, Fusarium culmorum, PCR, Surface plasmon resonance, Wheat

1. Introduction

The conventional approach to detect pathogenic *Fusarium* species in infected plants and grains involves the isolation of the fungus into axenic culture or culturing on selective media followed by identification based upon morphological characteristics (1). Recently, more reliable molecular methods for the detection of several *Fusarium* species have been reported based on PCR amplification of specific DNA regions (2–4). Measurement of PCR products obtained by conventional PCR methods is performed by gel electrophoresis that allows discrimination of different DNA fragments by their molecular weight. Despite these methods being simple and relatively inexpensive, they use mutagenic or potentially mutagenic labeling reagents. Moreover, PCR could provide byproducts arising from the amplification of nonspecific sequences

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with the same length of the desired fragment, leading to false-positive results. Therefore a hybridization assay is strongly recommended to confirm the selectivity of the reaction.

Surface plasmon resonance (SPR)-based DNA biosensors have been shown to be rapid, label-free, and selective tools for the detection of genetically modified organisms (GMOs) and of chemical and biological species, including fungal pathogens (5–9). By immobilizing a biological recognition molecule (probe) on the sensing surface, SPR biosensors provide a tool for real-time biospecific interaction analysis. The choice of a specific single-stranded DNA ligand allows rapid detection of the target DNA by hybridization with higher selectivity with respect to gel-electrophoresis analysis.

In this study, an SPR genosensor combined with PCR amplification has been used for the detection of *Fusarium culmorum* in pure fungal cultures as well as in wheat samples. *Fusarium culmorum* is a common pathogen of wheat involved in the etiology of Fusarium head blight (FHB), a worldwide disease causing total or partial ear premature senescence with consequent reduction of both crop yields and grain quality. In addition, *F. culmorum* can produce toxic secondary metabolites, mainly deoxynivalenol and zeralenone, which are mycotoxins harmful to humans and livestock (10–12).

2. Materials

Prepare all solutions using ultrapure water (e.g., produced by a Milli-Q system) and analytical grade reagents. Prepare and store all reagents at room temperature (unless indicated otherwise). Follow all waste disposal regulations when disposing waste materials.

2.1. Fungal Culture

1. Potato-dextrose-agar (PDA) (see Note 1).
2. Wickerham medium: 40 g glucose, 5 g peptone, 3 g yeast extract, 3 g malt extract, and water up to 1 L (see Note 2).

2.2. DNA Extraction from Fungal Cultures

1. Extraction buffer (SDS buffer): 200 mM Tris–HCl pH 7.8, 20 mM EDTA, 150 mM NaCl, 2% SDS, 1% β-mercaptoethanol, and 100 μg/mL proteinase K in water (see Note 3).
2. Phenol/chloroform/isoamylic alcohol (25:24:1, v/v/v).
3. Chloroform/isoamyl alcohol (24:1, v/v).
4. 3 M Sodium acetate, pH 5.2.
5. Absolute ethanol (ice cold).
6. 70% Ethanol (ice cold).

2.3. DNA Extraction from Wheat

1. Extraction buffer (CTAB buffer): 22 mM cetyltrimethylammonium bromide, 34 mM sarkosyl, 137 mM sorbitol, 22 mM EDTA, 1% cross-linked polyvinylpyrrolidone, 1.5 M NaCl in water (see Note 4).
2. Chloroform:isoamyl alcohol (24:1, v/v).
3. 5 M Potassium acetate.
4. Absolute ethanol (ice cold).
5. 70% Ethanol (ice cold).

2.4. DNA Quality and Quantification

1. DNA standard markers
2. 100 kb DNA ladder
3. 1× TAE buffer (Tris-Acetate-EDTA): 40 mM Tris, 20 mM acetic acid, and 1 mM EDTA (see Note **5**).
4. 1× TBE buffer (Tris-Borate-EDTA): 89 mM Tris, 89 mM boric acid, 2 mM EDTA (see Note **6**).
5. 0.7% Agarose gel.
6. 2% Agarose gel.
7. Ethidium bromide solution (see Note **7**).

2.5. PCR Amplification

1. Specific primers: 5′-ATG GTG AAC TCG TCG TGG C-3′ (Fc01F) and 5′-CCC TTC TTA CGC CAA TCT CG-3′ (Fc01R) (see ref. (13)).
2. Deoxynucleotide triphosphates (dNTPs).
3. Hotmaster™ Taq DNA polymerase.
4. Taq buffer with Mg^{2+}.
5. PCR centrifugal filter devices.

2.6. SPR Probes

1. 25-mer target probe (Fc01P): 5′-CTT TAC CCC TCT GTT ACT AAA CTA T-3′.
2. 25-mer reference probe (Ref01P): 5′-ATC TAA AAT GCT TCC AAA CAG CGG C-3′.
3. 5’ biotin-modified target probe (Fc01P) and reference probe (Ref01P).

2.7. SPR Analysis

1. Biacore® X apparatus (Biacore International AB, Uppsala, Sweden).
2. Biacore SA sensor chips (carboxymethylated dextran matrix pre-immobilized with streptavidin).
3. Running buffer (PBS-E300 buffer solution): 20 mM Na_2HPO_4 (pH 7.4), 0.1 mM EDTA, 300 mM NaCl in water.
4. Conditioning solution: 1 M NaCl and 50 mM NaOH in water.
5. Sensor surface regeneration solutions: from 2 to 50 mM NaOH.

3. Methods

3.1. Preparation of Fungal Cultures

1. Culture fungal strains at 25°C on potato-dextrose-agar (PDA) (see Note 8).
2. Take mycelium plugs from 7-day-old colonies on PDA. Inoculate mycelium into 100 mL of Whickeram liquid media and incubate at 25°C for 48 h on a rotary shaker (110 rpm) (see Note 9).
3. Collect the mycelium by filtration through Whatman No. 1 filter paper and lyophilize (see Note 10).

3.2. DNA Extraction from Fungal Cultures

Genomic DNA from fungal cultures is extracted according to the protocol described by Sambrook et al. (14).

1. Grind finely lyophilized mycelium (30 mg) and add 600 μL of hot SDS extraction buffer (see Note 11).
2. After incubation for 90 min at 65°C (see Note 12), add 700 μL of phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) solution. Mix thoroughly by inversion to form a complete emulsion. Centrifuge at 10,000 × *g* for 20 min at 4°C (see Note 13).
3. Transfer the supernatant solution from the aqueous phase (top) into a new microcentrifuge tube. Add 700 μL of chloroform/isoamyl alcohol (24:1, v/v) and perform the above procedure (step 2) (see Note 13).
4. Transfer the supernatant solution containing the DNA from the aqueous phase (top) into a new microcentrifuge tube. Add 0.1 volumes of 3 M sodium acetate at pH 5.2 and 2.5 volumes of cold absolute ethanol. Store mixture solution at −20°C for 2 h (see Note 14).
5. Centrifuge at 12,000 × *g* for 20 min at 4°C to allow DNA precipitation. Discard supernatant.
6. Wash the pellet with 1 mL of 70% cold ethanol and centrifuge at 12,000 × *g* for 10 min at 4°C. Discard supernatant. Repeat the washing with 1 mL of 70% cold ethanol, centrifuge and discard supernatant.
7. Dry the precipitated DNA under vacuum (see Note 15). Rehydrate the pellet with 100 μL sterile water and leave overnight at 4°C to allow complete dissolving.

3.3. DNA Extraction from Wheat

Genomic DNA from wheat grain samples is extracted according to the method described by Wilson et al. (15), with minor modifications.

1. Grind finely wheat kernels. Add 20 mL of hot CTAB buffer to 4 g of milled wheat into a 50 mL plastic conical tube (see Note 16).

2. Incubate at 65°C for 1 h with frequently vigorous shaking.
3. Add 6.7 mL (one-third volume) of 5 M potassium acetate along with 6.7 mL (one-third volume) of chloroform:isoamyl alcohol (24:1). Mix vigorously and store at –20°C for 20 min.
4. Centrifuge the mixture at 1,900 × *g* for 15 min.
5. Transfer 600 μL of supernatant solution containing the DNA from the aqueous phase (top) into a new microcentrifuge tube.
6. Add 1.2 mL of cold absolute ethanol. Mix and store at –20°C for 1 h (see Note 14).
7. Centrifuge at 850 × *g* for 10 min at 4°C to allow DNA precipitation. Discard supernatant.
8. Wash the pellet with 1 mL of 70% cold ethanol and centrifuge at 850 × *g* for 5 min at 4°C. Discard supernatant. Repeat the washing with 1 mL of 70% cold ethanol, centrifuge and discard supernatant.
9. Dry the precipitated DNA under vacuum (see Note 15). Rehydrate the pellet with 300 μL sterile water and leave overnight at 4°C to allow complete dissolving.

3.4. DNA Quality and Quantification

1. Prepare the 0.7% agarose gel by mixing 0.7 g agarose and 100 mL 1× TAE buffer, and then adding 2 μL of ethidium bromide solution (10 mg/mL). Stir to mix (see Notes 7 and 17).
2. Load 5 μL DNA standard markers and 10 μL DNA samples into separate wells.
3. Run the gel for 20–30 min at 60 mA.
4. Compare with DNA standard markers (see Note 18).

3.5. PCR Amplification

1. In a microcentrifuge tube prepare a PCR mixture (50 μL) containing: 1 ng fungal DNA or 30 ng of total DNA extracted from wheat samples, 100 μM of each deoxynucleotide triphosphate (dNTP), 100 nM of each oligonucleotide primer, and 2 U of Hotmaster™ Taq DNA polymerase in Hotmaster™ Taq buffer with Mg^{2+}.
2. Perform amplifications using following conditions: denaturation at 94°C for 2 min, then 40 cycles of 94°C for 50 s, 57°C for 50 s, and 72°C for 1 min, and a final extension at 72°C for 7 min followed by cooling to 4°C until recovery of samples (see Note 19).
3. In order to allow concentration and removal of primers and unincorporated nucleotides, purify PCR products by using Montage™ PCR centrifugal filter devices prior to SPR analysis according to the manufacturer's protocol (see Note 20).

3.6. PCR Product Quality

1. Prepare the 2% agarose gel by mixing 2 g agarose and 100 mL TAE buffer, and then adding 2 μL of ethidium bromide (10 mg/mL). Stir to mix (see Notes 7 and 17).
2. Screen PCR products by gel electrophoresis on 2% agarose gel, stained with ethidium bromide. Load 5 μL DNA ladder and 10 μL DNA samples into separate wells. Run the gel for 20–30 min at 60 mA.
3. Visualize using an UV transilluminator and compare with a 100 kb DNA ladder (see Note 18).

3.7. SPR Analysis (see Note 21)

1. Immobilize the biotinylated probes by injecting 100 μL of a 1 μM probe solution in PBS-E300 (flow rate of 5 μL/min), by means of biotin–streptavidin interaction (see Note 22).
2. Add 10 μL formamide to 40 μL of purified PCR products and then adjust the total volume to 100 μL with 2× PBS-E300 buffer (see Note 23).
3. Prior to SPR analysis, subject the mixture containing the PCR product to denaturation at 95°C for 5 min followed by 1 min cooling on ice.
4. Inject 50 μL of PCR denatured product into the SPR apparatus. All SPR measurements are carried out at 25°C. The flow rate is 5 μL/min and the injection time is 10 min (see Note 24).
5. Regenerate the sensor surface by performing short injections (up to 1 min) of 2 mM NaOH to 50 mM NaOH until complete recovery of the baseline RU value on both flow cells (see Note 25).

4. Notes

1. Suspend 39 g of powder in 1 L of distilled water. Heat until completely dissolved and sterilize in the autoclave at 121°C for 15 min.
2. Weight 40 g glucose, 5 g peptone, 3 g yeast extract, 3 g malt extract and transfer to 1-L graduated cylinder or a glass beaker. Add 900 mL distilled water and mix. Make up to 1-L with distilled water and mix. Transfer the liquid media in a flask and sterilize it in the autoclave at 121°C for 15 min.
3. For preparing 10 mL SDS extraction buffer add 2 mL of 1 M Tris–HCl pH 7.8, 400 μL of 1 M EDTA, 300 μL of 5 M NaCl, 2 mL of 10% SDS, and make up to 9.85 mL with distilled water. Mix gently. Sterilize in the autoclave at 121°C for 15 min. Prior to start DNA extraction, add 100 μL of β-mercaptoethanol and 50 μL of 20 mg/mL proteinase K to the buffer solution.

4. For preparing 100 mL CTAB buffer add 2.0 g cetyltrimethylammonium bromide, 34 mM sarkosyl, 137 mM sorbitol, 4 mL 0.5 M EDTA, 28 mL 5 M NaCl, 40 mL water and mix gently. Make up to 100 mL with distilled water. Sterilize in the autoclave at 121°C for 15 min. Prior to start DNA extraction, add 1 g polyvinylpyrrolidone to the buffer solution.
5. TAE buffer is commonly prepared as a 50× stock solution. A 50× stock solution can be prepared by dissolving 242 g Tris in water, adding 57.1 mL glacial acetic acid, and 100 mL of 500 mM EDTA (pH 8.0) solution, and bringing the final volume up to 1 l. Sterilize in the autoclave at 121°C for 15 min. This stock solution can be diluted 50:1 with water to make a 1× working solution. This 1× TAE buffer contain 40 mM Tris, 20 mM acetic acid, and 1 mM EDTA.
6. TBE buffer is commonly prepared as a 10× stock solution. A 10× stock solution can be prepared by dissolving 1 g NaOH, 108 g Tris, 55 g boric acid, 7.4 g EDTA and bringing the final volume up to 1 l. Sterilize in the autoclave at 121°C for 15 min. This stock solution can be diluted 10:1 with water to make a 1× working solution. This 1× TBE buffer contain 89 mM Tris, 89 mM boric acid, 2 mM EDTA.
7. Ethidium bromide may be a mutagen, carcinogen, or teratogen agent. Material should be handled with care. Preparation of solutions of ethidium bromide and any operations should be conducted in a fume hood to prevent inhalation. Gloves, lab coat, and eye protection should be worn at all times. When UV source is used in work with ethidium bromide, avoid exposing unprotected skin and eyes to UV source.
8. Fungal strains can be obtained from the ITEM collection (CNR Institute of Sciences of Food Production, Bari, Italy, http://www.ispa.cnr.it/Collection/). In our study, fungal strain included: 20 strains of *Fusarium culmorum* (ITEM 627, 628, 5991, 5996, 6221, 6229, 6234, 6249, 6255, 6260, 6264, 6267, 6268, 6269, 6270, 6625, 6627, 6628, 6629, 6630), 4 strains of *Fusarium graminearum* (ITEM 633, 4432, 4433, 4440), 2 strains of *Fusarium proliferatum* (ITEM 1725, 2620), and 1 strain of *Fusarium avenaceum* (ITEM 3409), *Fusarium poae* (ITEM 3725), *Fusarium cerealis* (ITEM 666), *Fusarium verticillioides* (ITEM 3993), *Fusarium subglutinans* (ITEM 3922), *Fusarium sambucinum* (ITEM 846), *Fusarium acuminatum* (ITEM 797), *Fusarium oxysporum* (ITEM 3867), *Aspergillus ochraceus* (ITEM 4537), *Aspergillus carbonarius* (ITEM 4864), *Aspergillus niger* (ITEM 4736) and *Penicillium expansum* (ITEM 7011). The strains are transferred from tube of fresh cultures stored at 4°C using a sterile loop on the PDA plate with a one point inoculum for the *Fusarium* species and a 3 points inoculum for the *Aspergillus* and *Penicillium* species.

The inoculums is made using sterile condition under a sterile laminar flow hood and sterilizing the loop under the flame.

9. Inoculum is made on sterile condition by inoculating the flask by mycelia plugs using a wire sterile loop. A 100 mL of Wickerham medium is put in 250 mL flask and sterilized at 121°C for 15′. For the sterility of the inoculum it is important to flame the neck and the rim of the flask before and after the mycelium inoculum. After 48 h incubation and before fungal culture filtration, in order to avoid bacteria contaminations it is important to check the purity of the culture by observing it under a light microscope.
10. Filtrate the culture using a Büchner funnel, a vacuum flask connected to a vacuum pump and a Whatman filter paper. After the liquid filtrated is completely passed through the paper, recover the mycelium in a sterile falcon using a spatula and store at –20°C.
11. Lyophilized mycelium is finely ground with a mortar and pestle. An aliquot is weighted in a microcentrifuge tube where hot (65°C) SDS buffer is added.
12. Place the microcentrifuge tube into a 65°C water bath for 90 min. Shake frequently the microcentrifuge tube by vigorous shaking.
13. Two phases separated by a white layer will be observed.
14. Carry out this step quickly to avoid heating of ethanol. An ice bath can be used during this operation.
15. SpeedVac® System has been found useful for this operation. Drying can be carried out also by a desiccator for 20–30 min. Do not allow the DNA to over dry or it will be hard to redissolve.
16. The Cyclotec™ Sample Mill with a 0.5-mm mesh screen was found suitable. Hot (65°C) CTAB buffer must be used.
17. Add agarose to 1× TAE in a 250 mL conical flask, stir vigorously to mix and heat the mixture in a microwave oven for approximately 2 min until the gel is completely melted, paying attention to avoid boiling. Allow to cool for approximately 5 min. Then add ethidium bromide and mix. Pour the gel slowly into the gel tray avoiding bubbles. Carefully place the comb. Allow the gel to set for at least 30 min at room temperature. Then place it in the gel tank and submerge with 1× TBE buffer to 2–5 mm depth.
18. In our study, gels were visualized under UV light on a ChemiDoc system (Bio-Rad Laboratories S.r.l., Segrate, Italy) and analyzed using a Bio-Rad Quantity One software. Presence of a highly resolved band indicates good quality DNA. Presence of smeared band indicates DNA degradation.

19. Tubes without DNA template were included in each experiment and used as blank control samples.
20. For *Fusarium culmorum*, the PCR amplified product should be 0.57 kb.
21. In our study, SPR analyses were performed by a Biacore® X apparatus (Biacore International AB, Uppsala, Sweden), using Biacore SA sensor chips (carboxymethylated dextran matrix pre-immobilized with streptavidin) for all experiments. The presence of two flow cells on the sensor chip allowed the immobilization of Fc01P on flow cell 1 (FC1), as probe for target DNA detection, and Ref01P on flow cell 2 (FC2), as a reference probe intended to investigate nonspecific interactions. PBS-E300 buffer solution was used as running buffer for immobilization of probes on sensor chips and for hybridization experiments. Running buffer should be filtered through 0.2 μm cellulose filter and daily degassed for 30 min under vacuum and stirring. In order to avoid bubble formation in the Biacore X microfluidic system, a cleaning step is weekly recommended by flowing 0.5% (w/v) SDS in water (BIA maintenance kit) for 22 min followed by 50 mM Glicyne–NaOH, pH 9.5 (BIA maintenance kit) for 3 min and running buffer for 10 min.
22. Prior to probe immobilization, condition sensor chips with three consecutive 1-min injections of a solution containing 1 M NaCl and 50 mM NaOH. In our experiments, the immobilization procedure based on streptavidin–biotin binding, resulted in signal shifts of $1{,}622 \pm 35$ RU ($n=7$) on flow cell 1 (FC1) for Fc01P probe (target DNA detection) and $1{,}568 \pm 64$ RU ($n=7$) on flow cell 2 (FC2) for Ref01P probe (reference).
23. In order to establish optimum hybridization conditions, PCR product solutions containing 20 mM Na_2HPO_4 (pH 7.4), 0.1 mM EDTA, NaCl at concentration from 0 to 600 mM, and denaturing agents (urea or formamide) at concentration from 0 to 30% were subjected to SPR analysis. The use of 10% formamide and 300 mM NaCl in the testing solution allowed a nearly fourfold increase of the hybridization efficiency with respect to reference conditions (i.e., PBS-E150 buffer solution: 20 mM Na_2HPO_4 [pH 7.4], 0.1 mM EDTA, 150 mM NaCl).
24. A real-time baseline correction was automatically performed during each measurement by subtracting the reference signal recorded in flow cell 2 (FC2) from the target signal recorded in flow cell 1 (FC1). The analytical signal was represented by the resonance unit (RU) shift measured during sample injection, i.e. the difference between the RU value recorded after washing the sensor surface following hybridization reaction

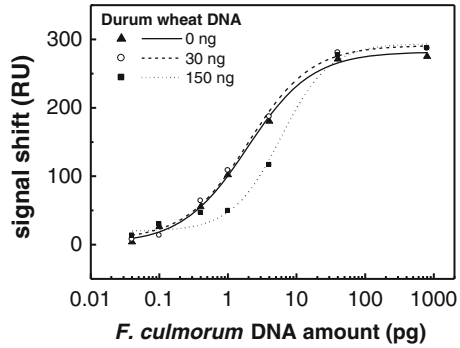

Fig. 1. Correlation between RU shift and the amount of starting *Fusarium culmorum* genomic DNA template (ITEM 627) in PCR, at different concentration of durum wheat DNA. All values were recorded in optimum experimental conditions (20 mM Na_2HPO_4 [pH 7.4], 0.1 mM EDTA, 300 mM NaCl, 10% formamide). Reproduced from ref. (7) with permission from Elsevier.

and the baseline RU value. In order to evaluate the matrix effect that could affect the SPR analysis of *F. culmorum* in wheat samples, PCR amplifications of different amount of genomic DNA of *F. culmorum* ranging from 0 to 800 pg were performed in presence of different amounts (0, 30, or 150 ng) of DNA of durum wheat buds grown under sterile conditions, and the relevant PCR products were detected by SPR biosensor (Fig. 1). Reliable results (similar to those obtained with pure culture) were observed only for sample with 30 ng durum wheat DNA. In the presence of 150 ng durum wheat DNA, RU shifts were significantly lower with respect to the corresponding values recorded with pure *F. culmorum* DNA, indicating an inhibitory effect of the matrix on PCR amplification. Detection limits of 0.05 pg (corresponding to a signal shift of 10.1 RU) and 0.06 pg (corresponding to a signal shift without correction for blank of 17.4 RU) of *F. culmorum* DNA template were observed in the absence and presence of 30 ng of durum wheat DNA, respectively.

25. The sensor surface is regenerated under strong alkaline conditions (pH > 11) allowing at least 75 hybridization cycles with a sensitivity loss lesser than 15%. NaOH at higher concentrations is used for sensor surface regeneration after a large number of hybridization cycles.

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References

1. Nirenberg HI (1981) A simplified method for identifying Fusarium spp. occurring on wheat. Can J Botany 59:1599–1609
2. Edwards SG, Pirgozliev SR, Hare MC, Jenkinson P (2001) Quantification of trichothecene-producing Fusarium species in harvested grain by competitive PCR to determine efficacies of fungicides against fusarium head blight of winter wheat. Appl Environ Microbiol 67:1575–1580
3. Yli-Mattila T, Paavanen-Huhtala S, Jestoi M, Parikka P, Hietanieme V, Gagkaeva T, Sarlin T, Haikara A, Laaksonen S, Rizzo A (2008) Real-time PCR detection and quantification of Fusarium poae, F. graminearum, F. sporotrichioides and F. langsethiae in cereal grains in Finland and Russia. Arch Phytopathol Plant Protection 41(4):243–260
4. Brunner K, Mach RL (2010) Quantitative detection of fungi by molecular methods: a case study on Fusarium. In: Voigt K, Gherbawy Y (eds) Molecular identification of fungi. Springer, Berlin, Heidelberg, pp 93–106
5. Homola J (2008) Surface plasmon resonance sensors for detection of chemical and biological species. Chem Rev 108:462–493
6. Homola J (2006) Surface plasmon resonance based sensors. Springer, Berlin, Heidelberg, p 251
7. Zezza F, Pascale M, Mulè G, Visconti A (2006) Detection of Fusarium culmorum in wheat by a surface plasmon resonance-based DNA sensor. J Microbiol Methods 66:529–537
8. Skottrup P, Hearty S, Frøkiær H, Leonard P, Hejgaard J, O'Kennedy R, Nicolaisen M, Justesen AF (2007) Detection of fungal spores using a generic surface plasmon resonance immunoassay. Biosens Bioelectron 22:2724–2729
9. Manera MG, Spadavecchia J, Leone A, Quaranta F, Rella R, Dell'Atti D, Minunni M, Mascini M, Siciliano P (2008) Surface plasmon resonance imaging technique for nucleic acid detection. Sens Actuators B Chem 130:82–87
10. Wagachaa JM, Muthomib JW (2007) Fusarium culmorum: infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. Crop Prot 26:877–885
11. Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals-a review. Plant Pathol 44:207–238
12. Logrieco A, Bottalico A, Mulé G, Moretti A, Perrone G (2003) Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur J Plant Pathol 109:645–667
13. Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW, Joyce D (1998) Detection and quantification of Fusarium culmorum and Fusarium graminearum in cereals using PCR assays. Physiol Mol Plant Pathol 53:17–37
14. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York
15. Wilson A, Simpson D, Chandler E, Jennings P, Nicholson P (2004) Development of PCR assays for the detection and differentiation of Fusarium sporotrichioides and Fusarium langsethiae. FEMS Microbiol Lett 233:69–76