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A practicable detection system for genetically modified rice by SERS-barcoded nanosensors

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ABSTRACT

Since the global cultivation of genetically modified crops constantly expands, it remains a high demand to establish different ways to sort food and feed that consist or contain genetically modified organisms. Surface-enhanced Raman scattering (SERS) spectroscopy is a flexible tool for biological analysis due to its excellent properties for detecting wide varieties of target biomolecules including nucleic acids. In the present study, a SERS-barcoded nanosensor was developed to detect *Bacillus thuringiensis* (Bt) gene-transformed rice expressing insecticidal proteins. The barcoded sensor was designed by encapsulation of gold nanoparticles with silica and conjugation of oligonucleotide strands for targeting DNA strands. The transition between the *cry1A(b)* and *cry1A(c)* fusion gene sequence was used to construct a specific SERS-based detection method with a detection limit of 0.1 pg/mL. In order to build the determination models to screen transgene, a series mixture of Bt rice and normal rice were prepared for SERS assay, and the limit of detection was 0.1% (w/w) transgenic Bt rice relative to normal rice. The sensitivity and accuracy of the SERS-based assay was comparable with real-time PCR. The SERS-barcoded analytical method would provide precise detection of transgenic rice varieties but also informative supplement to avoid false positive outcomes.

1. Introduction

Bacillus thuringiensis (Bt), a Gram-positive bacterium, produces crystals of proteinaceous δ-endotoxins during sporulation. The insecticidal crystal proteins (Cry proteins) are encoded by *cry* genes (Hofte and Whiteley, 1989). With the development of plant genetic engineering, genes expressing Bt insecticidal crystal protein can be introduced into plant for insect control and several species of Bt crops have become commercially available worldwide. Commercialization of Bt crops has significantly reduced the use of synthetic insecticides (Ferre and Van Rie, 2002). Although the environmental impacts of Bt crops appear to be positive (Brookes and Barfoot, 2006; Tu et al., 2000), the coexistence of genetically modified (GM) plants with conventional and organic crops has raised worldwide significant concern, which leads to the adoption of regulations requiring safety test of GM foods produced for human consumption (Konig et al., 2004).

Testing on genetically modified organisms (GMOs) in food and feed is often done by employing DNA-based techniques such as Southern blot (Yang et al., 2005), polymerase chain reaction (PCR) (Hiroshi et al., 2005), DNA microarrays (Morisset et al., 2008), quick test strip (Emanuela et al., 2000), GM metabolite map and biosensor (Bai et al., 2007; Carpini et al., 2004; Mannelli et al., 2003), as well as protein-based techniques like ELISA and Western blot (Holst-Jensen, 2009). The methods based on gene sequence detection are not only highly sensitive, but relatively more accurate and reliable. Therefore, routinely analytical methods of GMOs are mainly DNA-based microarrays or PCR (Morisset et al., 2008; Yang et al., 2005; Hiroshi et al., 2005). At present, the analysis of rice (*Oryza sativa*) and rice products for Bt transgenic sequence mainly relies on PCR screening combined with specific methods for further characterization (Dietrich et al., 2006). Among these methods, real-time PCR is often preferred because of its high specificity and stability both in qualitative and quantitative analysis. In most real-time PCR detection methods, special thermo cycler is required, which can monitor the fluorescence during reaction. The fluorescent probes are used to detect PCR products during each cycle of amplification process (Holland et al., 1991). To detect and identify single gene sequence from whole genome by fluorescence, DNA must be labeled by fluorophores, organic or inorganic, fluorescent dyes or semiconductor quantum dots (QDs) (Han et al., 2001), and these fluorescent labels must achieve large enough fluorescence quantum yields and distinguishable spectral properties, or the spectral overlapping and fluorophore photobleaching will hinder the analytical performance of the test (Kneipp et al., 1999). An alternative approach is surface-enhanced Raman scattering (SERS) spectroscopy, an emerging technique for detecting and recognizing a single DNA strand through nanoparticles functionalized with

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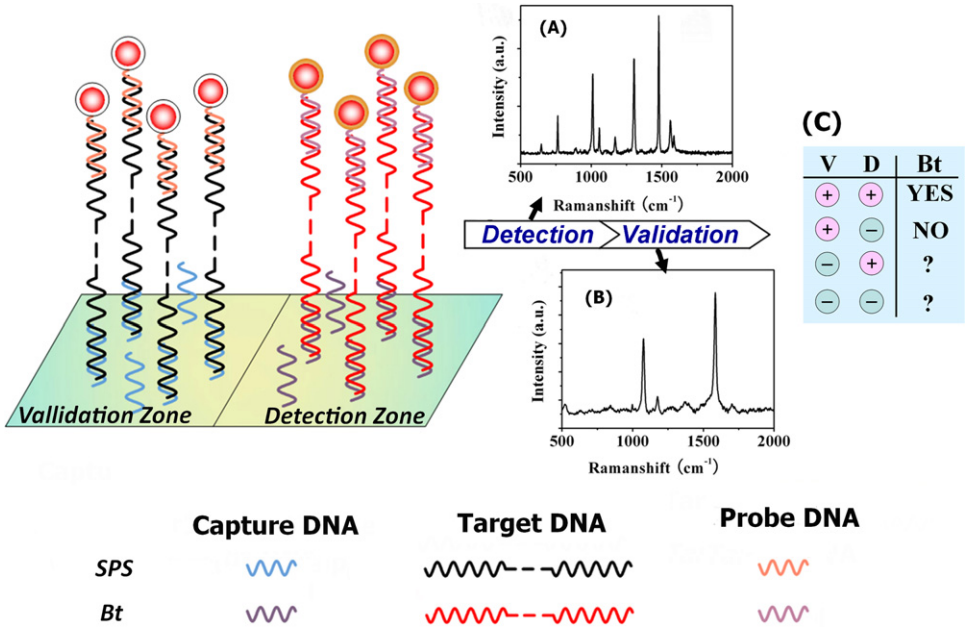

Fig. 1. Schematic illustration of Bt gene detection procedure using SERS-barcoded nanotags. (A) Representative SERS spectrum of *Bt*; (B) representative SERS spectrum of *SPS*; (C) table of detection results and results determination.

oligonucleotides and Raman labels (Kang et al., 2010; Storhoff et al., 2004).

SERS is a well-established analytical tool which has the potential to reach the high sensitivity as fluorescence spectroscopy and keep the abundant structural information content of Raman spectroscopy (Kneipp et al., 1999). Raman scattering-based techniques attract extensive interest among life science applications (Shafer-Peltier et al., 2003; Qian et al., 2008a,b; Graham et al., 2008). The utilization of SERS-based techniques in targeting specific DNA and RNA sequences is by means of combination of gold and silver nanoparticles and Raman-active molecules (Cao et al., 2002; Bell and Sirimuthu, 2006; Qian et al., 2008a,b; Lim et al., 2010). Most Raman molecules are commercially available, and each spectrum is specific, leading to the development of non-overlapping and anti-photobleaching probes for gene detection advantageous over fluorescence.

SERS-barcoded particles are amenable to high-throughput and multiplex DNA screening (Seydack, 2005). The barcoded particles are coated with a suitable protective shell, usually silica or polymer, with a layer of Raman-active molecules embedded between the shell and metal particle. The shell confines the Raman codes around the metal particle, and protects them from leaching out, while providing high colloidal stability and a feasible surface for biomolecular conjugation (Guerrero-Martínez et al., 2010). In addition, these SERS particles carry barcodes with narrow spectral peaks, which limits spectral overlap to a minimum level and offers picomolar even femtomolar sensitivity in biochemical analysis (Gong et al., 2007). Furthermore, the SERS-barcoded tags which contain Raman-active molecules adsorbed to the metal surface are insensitive to photobleaching and coated with silica making them fairly low cytotoxic (Guerrero-Martínez et al., 2010).

In this study, we designed a transgenic gene nanosensor using SERS-barcoded nanoparticles as spectroscopic tags to detect Bt gene in rice seeds. The SERS-barcoded nanoparticles were fabricated with a golden core for optical enhancement, a layer of Raman reporter molecules absorbed onto the surface of gold core for spectroscopic barcoding, and a silica shell for protection and functionalization. After conjugated with specific oligonucleotides, the SERS-barcoded tags were ready for barcoding target gene. The core–shell structure is highly suitable to multiplexed target detection at single particle level. With the SERS-barcoded nanoparticles, a number of genes can be examined at low abundance especially in complex biochemical environment such as DNA hybridization solution. The Bt genes usually used in rice are *cry1Ab*, *cry1Ac*, and fused *cry1Ab/cry1Ac* (Cheng et al., 1998; Ramesh et al., 2004). Because of the limitation of allowable GMO source, we set transition between the *cry1A(b)* and *cry1A(c)* fusion gene sequence, namely *Bt* as our only target gene, and *SPS* (gene of rice sucrose phosphate synthase) as interior reference gene of rice (Jiang et al., 2009) to set up a transgenic products nanosensor based on SERS-barcoded tags (see Fig. 1). Capture DNA of *Bt* and *SPS* anchored on glass substrate at each corresponding testing zone was used to match relevant gene fragments. The fixed fragments were barcoded with SERS tags by hybridization to complementary sequences. Finally, the testing results were decoded by reading the SERS spectra at the detection zone and verified testing effectiveness at the validation zone. At the same time, through analyzing the Raman intensity, the amount of transgenic fragments in samples was estimated.

2. Material and methods

2.1. Chemicals and materials

All chemicals and solvents were of analytical grade and used as received without further purification. 4-Aminobenzenethiol (ABT) was purchased from J&K Chemical Ltd.; *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS), sodium silicate solution, (3-glycidyloxy propyl)trimethoxysilane (GPTMS) and tetraethyl orthosilicate (TEOS) were from Sigma-Aldrich; 2,2′-dipyridyl (DP) and other relevant reagents were from Sinopharm Chemical Reagent Co. Ltd.

Primers of *SPS* (SPS-F1 and SPS-R1), primers of *Bt* (Bt-F2 and Bt-R2), capture DNA of *SPS* (SPS-C1), capture DNA of *Bt* (Bt-C2), probe DNA of *SPS* (SPS-P1), and probe DNA of *Bt* (Bt-P2) were synthesized by AuGCT biotechnology Co., Ltd. (Beijing, China). The oligonucleotide sequences are listed in Table 1. The Bt rice seeds were kindly provided by National Key Laboratory of Crop Genetic Improvement, and the non-transgenic rice seeds were purchased

Table 1
Oligonucleotide sequences used in this work.

Gene	Description	Sequence (5′-3′)	Product (bp)
SPS	SPS-F1	F1: TTG CGC CTG AAC GGA TAT	277
	SPS-R1	R1: GGA GAA GCA CTG GAC GAG G	
	Capture 1	C1: TGA AAG ATA TCC GTT CAG GCG CAA A_{10}–NH_2	–
	Probe 1	P1: NH_2–A_{10} GGA GAA GCA CTG GAC	–
	Bridge 1	B1: GTC CAG TGC GTC CAG TGC	–
Bt	Bt-F2	F2: GAA GGT TTG AGC AAT CTC TAC	300
	Bt-R2	R2: CGA TCA GCC TAG TAA GGT CGT	
	Capture 2	C2: TTG GTA GAG ATT GCT CAA ACC TTC A_{10}–NH_2	–
	Probe 2	P2: NH_2–A_{10} CGA TCA GCC TAG TAA	–
	Bridge 2	B1: TTA CTA GGC TTA CTA GGC	–

from a local seed company, Wuhan, China. DNA was extracted with *EasyPure* plant genomic DNA kit (TransGen Biotech, Beijing, China), amplified with *pEASY*-T1 cloning kit and 2 × *EasyTaq* PCR SuperMix (TransGen Biotech, Beijing, China) and purified with *EasyPure* quick gel extraction kit (TransGen Biotech, Beijing, China). Detailed sequence information of *Bt* and *SPS* is available in GenBank database of the National Center for Biotechnology Information (NCBI).

Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used throughout the experiment.

2.2. Instrumentation

All Raman spectra were performed using invia Raman spectrometer (Renishaw, UK) equipped with co-focusing microscope (Leica, German). The samples were excited under a He–Ne laser (633 nm) with laser power of approximately 10 mW. The band of a silicon wafer at 520 cm^{-1} was used to calibrate the spectrometer. Unless otherwise mentioned, the SERS spectra were acquired with 10 s exposure and one time accumulation.

The UV–vis absorption spectra were obtained on Nicolet Evolution 300 UV–vis spectrometer (Thermo Nicolet, America). Transmission electron microscopy (TEM) images were acquired by a JEM-2010 transmission electron microscope (JEOL, Japan). Hydrodynamic diameters were measured with a Zetasizer Nano ZS90 DLS system (Malvern, England). The PCR experiments were carried out on a Roche LightCyler (Roche, America), and Real-time PCR was performed in a Bio-Rad iCycler (Roche, America). PCR products were separated on DYCP-32B electrophoresis apparatus (Beijing Liuyi, China).

2.3. Synthesis of SERS-barcoded tags

Au nanoparticles (Au NPs) of approximately 32 nm in diameter serving as gold core were synthesized according to a traditional citric acid reduction method described in literature (Frens, 1973). The colloids were centrifugated at 6000 rpm to remove excess ions and resuspended in water. The SERS-barcoded nanoparticles were packaged following the literature procedures (Guerrero-Martínez et al., 2010; Lu et al., 2002). Raman reporter (DP or ABT) was added into the Au colloids solution under vigorous stirring to a final concentration of 0.05 μM and equilibrated for 30 min. Then these gold colloids were directly coated with conformal shells of amorphous silica using the sol–gel process.

The obtained SERS-barcoded nanoparticles were dispersed in 10 mL toluene. 20 μL GPTMS was added to the solution under stirring and refluxed for 6 h at 120 °C (Li et al., 2011). After cooling, the mixture was centrifuged for 15 min, washed with ethanol several times to remove by-products and redispersed in water. The nanoparticles were capped with amino modified probe DNA in the presence of EDC and NHS at pH 8.5. Correspondingly, DP embedded SERS-barcoded nanoparticles were capped with Bt-P2, and ABT embedded SERS-barcoded nanoparticles were SPS-P1. These SERS-barcoded tags were centrifugated respectively at 6500 rpm for 15 min to get rid of unbounded DNA. The precipitates were dispersed in 10 mM PBS and stored at 4 °C.

2.4. Preparation of SERS-barcoded nanosensor

Capture DNA was immobilized onto functionalized glass slide treated via glutaraldehyde method. 0.1 mM amino-capped SPS-C1 (in a 10 mM PBS solution with 0.2 M NaCl) was spotted onto the validation zone of functionalized glass slide, and amino-capped Bt-C1 was spotted onto the detection zone. The slide was kept in a humidity chamber for 12 h, washed with ultrapure water to remove free capture DNA and dried, passivated the rest region of the slide surrounding the test spots by succinic anhydride solution for 4 h. At last, the slide was and washed and dried again.

2.5. Transgenic gene detection procedure

In a typical determination procedure, target sequences were mixed with 55 °C preheated hybridization solution and kept at 95 °C for 3 min, 0 °C for 5 min, subsequently. Immediately, pretreated target sequences mixed with 0.8 M NaCl PBS buffer of SERS-barcoded tags were transferred to the spots and kept in a humidity chamber at 40 °C for 1 h to effectively hybridize with the overhanging period of the targets. Then the slide was washed with 0.8 M NaCl PBS buffer to eliminate nonspecifically bound sequences, subsequently rinsed with ultrapure water and dried. The spots were scanned by Raman spectroscopy and each result was averaged on 8 times parallel determination. In the case of plants material, the targets were raw PCR products.

For specificity tests, PCR products from non-transgenic plants were used as blank control. For sensitivity tests, a serial dilution of purified PCR products from GM plants stepwise tenfold diluted with TE buffer were used as detection strands. But in field-tests, seed samples of mixed Bt rice and normal rice were cultivated in house and the up-ground part was extracted genomic DNA for SERS test.

3. Results and discussion

3.1. Characterization of SERS-barcoded tags

As depicted in Fig. 2, absorption spectra of SERS-barcoded nanoparticle (Au@Si NPs, Fig. 2A) showed a 3 nm red shift to the absorption of original Au NPs at 527 nm which was consistent with the local plasmon resonance of Au NPs with an average diameter of 32 nm. The red shift was primarily attributed to the silica coating. Small organic compounds such as DP and ABT used in our experiment, may not cause apparent red shift at inferior concentration (Kustner et al., 2009). Red shift occurs when the surrounding medium changes, because the plasmon resonance frequencies are

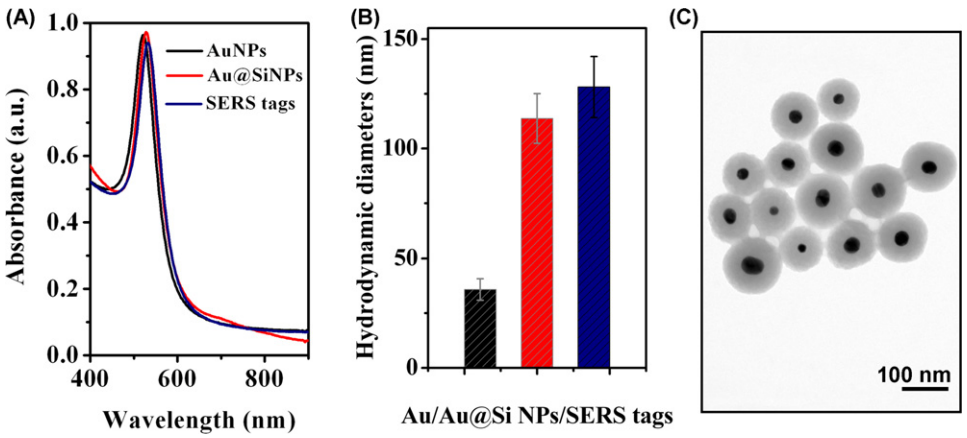

Fig. 2. Characteristic of SERS-barcoded tags. (A) UV–vis spectra of Au NPs, Au@Si NPs and SERS tags; (B) the corresponding hydrodynamic diameters of Au NPs (black column), Au@Si NPs (red column) and SERS tags (blue column); (C) TEM of SERS tags coated with silica shells. Error bars: the standard deviation of three measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

dependent on the refractive index of the surrounding medium and silica has a higher refractive index ($n = 1.57$) than water ($n = 1.33$) (Lu et al., 2002). Under the same reaction condition including reaction temperature, time, pH and concentration, the absorption spectra of Au@Si NPs with and without Raman reporters showed little change to each other (Jang et al., 2004), but the hydrodynamic diameters were increased to ~114 nm (Fig. 2B). When conjugated with probe DNA, the Au@Si tags exhibited plasmon resonance absorption at 532 nm, while their hydrodynamic diameters were enlarged to 128 nm (Fig. 2B). This phenomenon might be attributed to the surface coverage of oligonucleotides enlarging particle diameters in water solution. The morphology of the core–shell nanotags was confirmed by TEM (Fig. 2C), and the average shell thickness was about 45 nm coincident with hydrodynamic diameter measurement.

3.2. SERS characteristics of barcoded nanosensor

Between 500 and 2000 cm^{-1}, the SERS spectra of Au@Si NPs without Raman codes were as flat as the primal Au NPs (Fig. 3A, a and b), indicating the cleanliness of obtained Au@Si NPs in favor of the application in SERS-barcoding. The characteristic Raman spectra of ABT and DP on the surface of Au NPs are given in Fig. 3 (A, line c and d). Strong peaks centered at 1578 and 1076 cm^{-1} are assigned to C=C stretch and C=S stretch of ABT (Zhou et al., 2006; Wang et al., 2007). For DP molecules, the main characteristic Raman bands appear at 765 cm^{-1} assigned to in plane ring deformation, 1011 cm^{-1} assigned to ring breathing, 1305 cm^{-1} assigned to C—C inter ring stretch and 1478 cm^{-1} assigned to C=N stretch and in plane C—H deformation (Joo, 2004; Zhu et al., 2011). These small organic molecules gave rise to simple and clear Raman spectra, so in the following section, the Raman band of ABT at 1578 cm^{-1} was selected as the quantitative peak of *SPS*, while the band of DP at 1478 cm^{-1} was regard as the quantitative peak of *Bt*.

The schematic design of *Bt* detection employing SERS nanosensor is shown in Fig. 1. To verify the detection process from DNA ligation, we conducted some validation contrast test, and the results are shown in Fig. 3B. For validation contrast test, when 10 ng/mL targets were tested in the presence of probes but in the absence of capture DNA, the Raman signal was close to zero, because no targets could be selectively fixed and readily washed away. In the case of probe DNA absence but captures and targets presence, no obvious Raman peak was observed because the probe DNA was key factor matching with target DNA and barcoding target DNA with SERS labels. Since the missing link of probes, no significant Raman signals could be detected. Also, low signals observed in the presence of captures and probes but in the absence of targets, probably arise from nonspecific adsorption of SERS-barcoded tags. In addition, nonspecific adsorption became severe when *SPS* sequences were used as parallel references blended with *Bt* to test the detection specificity. Long stranded DNA are prone to tangle with each other, leading to the nonspecific adsorption. To cut down the nonspecific recognition of non-target disturbance DNA sequences, following experiments were carried out to improve the performance of SERS-barcoded nanosensor.

3.3. Optimization of transgenic gene detection

DNA hybridization in this SERS based assay was carried out in solid phase where fixed sequence was not in liquid phase and thus its concentration could not be computed accurately. On such occasion, hybrid rate primarily relies on the dissociative DNA length and concentration. At the beginning of hybridization, the hybridization dynamics of fixed DNA with dissociative DNA is close to the

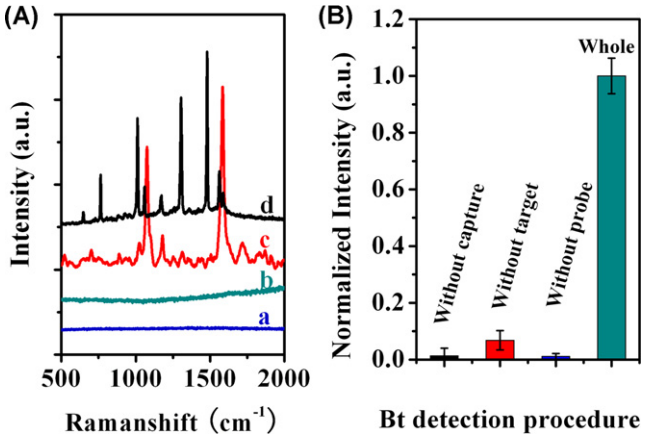

Fig. 3. (A) SERS spectra of Au NPs (a), Au@Si NPs (b), SERS tags for *SPS* (c) and SERS tags for *Bt* (d). (B) SERS intensity variation in the case of without capture DNA (black column), without target DNA (red column), without probe DNA (blue column) and the completed case (green column). The target DNA concentration was 10 ng/mL. Error bars showed the standard deviation of three measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

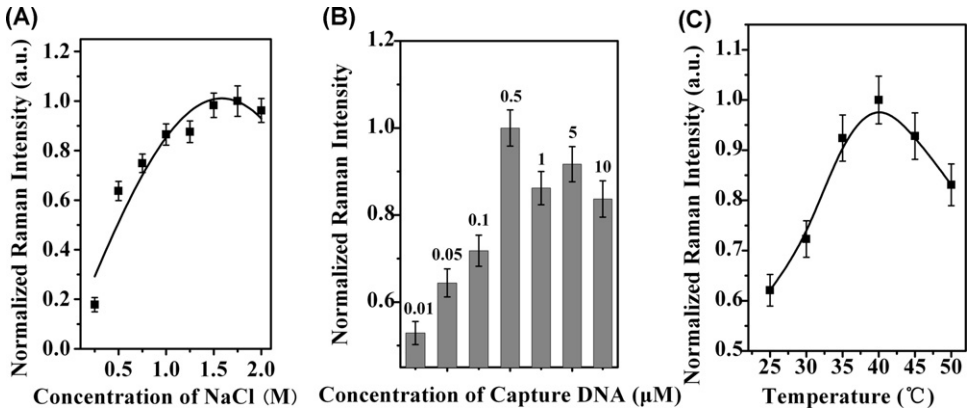

Fig. 4. Effect of NaCl concentration (A), capture DNA concentration (B) and hybridization temperature (C) on the sensitivity of the SERS-barcoded nanosensor. The target DNA concentration was 10 ng/mL. Error bars were calculated based on the standard deviation of eight measurements.

hybridization dynamics of dissociative DNA themselves. As the hybridization processing, dissociative DNA annealing takes place along with hybridizing with fixed DNA, hence the single stranded DNA keeps on declining. Besides DNA sequence length and concentration, other external factors such as temperature and ionic strength could also enhance the hybrid rate under suitable conditions (Holland et al., 1991). In order to improve the hybrid rate and detection accuracy, 0.8 M NaCl (Fig. 4A) was used to maintain the optimum ionic strength for DNA hybridization, 0.5 μM capture DNA (Fig. 4B) was employed to catch the target DNA in our research, and the optimized hybridization was performed at 40 °C (Fig. 4C) in the following experiments.

3.4. Sensitivity of SERS-barcoded nanosensor

To analyze the sensitivity of the SERS-barcoded nanosensor, purified *Bt* PCR products were sequentially ten-fold diluted with 0.1 × TE buffer and barcoded with SERS tags for Raman test. The results in Fig. 5A illustrate that the increase of Raman intensity was dependent on the increased *Bt* concentration. In the present SERS assay, also referred to sandwich assay on the other side, one end of the PCR product in the unknown was bound to the capture DNA site, and the opposite end was connected with probe DNA which was marked with SERS codes. This process was the barcoding section. Then the sample was scanned by Raman spectrum, which was the decoding section. Through analyzing Raman peak position and intensity, the target DNA was recognized and the amount of DNA was measured based on sequence-specific bounding. By analyzing the peak intensity at 1478 cm^{-1}, the change of *Bt* concentration could be quantitatively evaluated (Fig. 5B). From 1.0 pg/mL to 10 ng/mL, the logarithm of *Bt* concentration linearly related to the SERS intensity, and the current optimized detection limit of SERS-barcoded nanosensor reached 0.1 pg/mL, which was comparable with real-time PCR method (Dietrich et al., 2006; Su et al., 2011). At such a low concentration level, the SERS intensity was still visible and over three times of blank control. For a 300 bp DNA segment, 5 μL of 0.1 pg/mL sample amount contains about 30,000 target molecules. Each test spot area is nearly 1 mm^2 in size so there is 0.06 molecules every 1 μm^2. With narrow-field laser illumination, less than one particle was examined in one field of view 10 μm^2 sampling area. The number of nanoparticles was proportional to the fixed target molecules. At least 200 small organic molecules were absorbed onto each particle, whick could provide distinct Raman signal. The evidence proved that the SERS-based measurement has been operated at the single-particle level.

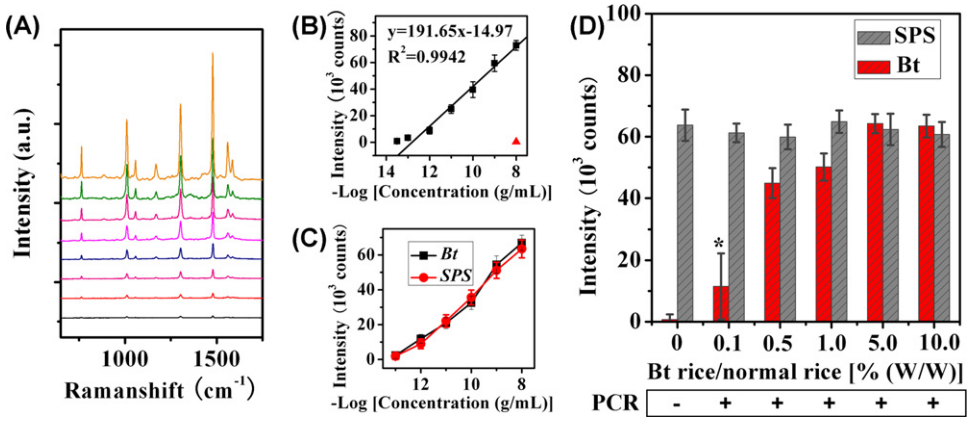

Fig. 5. (A) SERS spectra of the nanosensor for detecting different concentrations of *Bt* (from bottom to top: 0.0 pg/mL, 0.05 pg/mL, 0.1 pg/mL, 1.0 pg/mL, 10 pg/mL, 100 pg/mL, 1.0 ng/mL, 10 ng/mL, nethermost black line: blank control by using *SPS*). (B) Raman intensity response to different concentrations of *Bt* (inserts: linear equation and relational coefficient, red triangle: blank control). (C) Raman intensity response to different concentrations of *Bt* (black spots and line) and *SPS* (red spots and line) in the mixture of PCR products of *Bt* and *SPS*. (D) Normalized Raman intensity and real-time PCR results in the presence of different amount of Bt rice relative to normal rice. Error bars were calculated based on the standard deviation of eight measurements. *Comparison of obtained result with real-time PCR. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Another serial dilution was prepared for sensitivity testing (Fig. 5C) by means of sequentially ten-fold diluting a mixture of PCR products from Bt gene (10 ng/mL) and SPS gene (10 ng/mL). The diluted mixtures were tested both on detection zone and validation zone (Fig. 1, insert A and insert B). The detection zone employed DP embeded SERS tags, while validation zone met to ABT embedded SERS tags. After proper rinse, both two zones were scanned by Raman spectrograph. Although all the SERS intensity became a little lower than previous test, the variation tendency agreed well with experimental result described above (Fig. 5B and C). Despite a few disturbances from *SPS*, the detection limit of the SERS-barcoded nanosensor for Bt gene reached 1.0 pg/mL almost as sensitive as before. The results ensured that the SERS-barcoded nanosensor could be used to analyze composite sample for practical detection of Bt rice.

3.5. Transgenic gene detection in rice

Commercially available rice samples mixed with different amount of Bt rice were also indoor planted and extracted genomic DNA for PCR amplification. Primers of *Bt* and *SPS* were used to amplify target gene. *Bt* PCR products were tested in detection zone, and *SPS* PCR products were tested in validation zone. The Raman spectrum acquired from detection zone indicated whether the PCR products in unknown contained Bt gene fragments, and from detection zone demonstrated whether the test results were reliable (Fig. 1, insert table). *SPS*, a proved suitable endogenous reference gene for GM rice detection (Jiang et al., 2009), here is used as an internal reference in the optimized SERS systems to ensure the results reliable. Rice samples mixed with different amount of Bt rice were determined with SERS-barcoded nanosensor (Fig. 5D). Zones were exposed to positive and negative samples as judged by a reference standard. From 0.5% (w/w) to 10% (w/w), the test results illustrated that after read by Raman spectrograph each gene could be decoded and identified. Ultimately, a mixture of 19.98 g conventional rice and 0.02 g of Bt rice resulting in a 0.1% (w/w) sample was used to check the limit of the SERS-based method. The test result of 0.1% (w/w) sample exhibited large fluctuation, elucidating that the present method reached the detection limit in rice sample determination. This could be ascribed to the low target sample abundance which aroused the sampling randomness. But in an effective sampling, combined with PCR amplification one would be able to use the detection system to identify other exogenetic gene transferred to crops even when there are mutants blending in the transgenic plants.

4. Conclusions

Regional authorization of GMO has led to the foundation of supervisory committee to protect consumer's preference. With the help of PCR, our group has conducted a complete transgene detection procedure for rice samples base on SERS-barcoded nanosensors. The new system permits the determination of 0.1 pg/mL transgenic Bt gene and the distinguishment of 0.1% (w/w) Bt rice from normal rice impure samples. It has demonstrated that the accuracy and limit of detection were comparable with real-time PCR while analyzing Bt transgene in rice sample materials. Furthermore, DNA hybridization studies indicate that the SERS-based method has the potential to recognize target gene at single-bead level. This spectral barcoding technology is expected to bring in new opportunities in gene analytic studies and high-throughput DNA screening, shining a scientific spotlight on little-studied SERS-based gene detection to refine the much-debated genetically modified organisms.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2012.01.029.

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