Original Article | Journal of Biosystems Engineering

J. Biosyst. Eng. 43(4):394-400. (2018. 12)
https://doi.org/10.5307/JBE.2018.43.4.394

eISSN : 2234-1862
pISSN : 1738-1266

Evaluation of SERS Nanoparticles to Detect *Bacillus cereus* and *Bacillus thuringiensis*

Jeehwa Hong[1,2], Jianwei Qin[1], Jo Ann S. Van Kessel[1], Mirae Oh[1], Sagar Dhakal[1], Hoonsoo Lee[3], Chansong Hwang[1], Diane E. Chan[1], Dongho Kim[2], Hyunjeong Cho[2]*, Moon S. Kim[1]*

[1]*Environmental Microbial and Food Safety Laboratory, Agricultural Research Service, US Department of Agriculture, MD 20705, USA*
[2]*Experiment Research Institute, National Agricultural Products Quality Management Service, Gimcheon 39660, Korea*
[3]*Dept. of Biosystems Engineering, Chungbuk National University, Cheongju 28644, Korea*

Received: September 28th, 2018; Revised: November 9th, 2018; Accepted: November 11th, 2018

Abstract

Purpose: This research evaluated five types of nanoparticles to develop a surface-enhanced Raman spectroscopy (SERS) method for the rapid detection of two *Bacillus* species (*Bacillus cereus* and *Bacillus thuringiensis*) that are commonly found on fresh produce, which can cause food poisoning. **Methods:** Bacterial concentrations were adjusted to a constant turbidity, and a total of 30 μL of each *Bacillus* cell suspension was prepared for each nanoparticle. A point-scan Raman system with laser light source of wavelength 785 nm was used to obtain SERS data. **Results:** There was no qualitative difference in the SERS data of *B. cereus* and *B. thuringiensis* for any of the five nanoparticles. Three gold nanoparticles, stabilized in either citrate buffer or ethanol, showed subtle differences in Raman intensities of two *Bacillus* species at 877.7 cm^{-1}. **Conclusions:** Among the three types of nanoparticles, the gold nanoparticles stabilized in citrate buffer showed the lowest standard deviation, followed by gold nanoparticles stabilized in ethanol. This result supports the potential application of gold nanoparticles for SERS-based detection of *B. cereus* and *B. thuringiensis*.

Keywords: *Bacillus cereus*, *Bacillus thuringiensis*, Nanoparticle, Point-scan Raman, SERS

Introduction

Bacillus cereus, Bacillus anthracis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus thuringiensis, and Bacillus weihenstephanensis are six *Bacillus* species that have been identified as agents of foodborne illness (Ankolekar et al., 2009). *B. cereus* is a gram positive, spore-forming, and facultative anaerobic bacterium found in soil, contaminated irrigation water, fresh raw vegetables, and cooked rice (Organji et al., 2015). Infection due to *B. cereus* may result in diarrheal and emetic syndromes, depending on the degree of expression of the enterotoxin gene (Li et al., 2016). *B. thuringiensis* is a spore-forming bacterium and is closely related to *B. cereus*. Unlike *B. cereus*, *B. thuringiensis* has parasporal crystal bodies that are encoded by the cry genes (Vilas-Bôas et al., 2007).

Worldwide outbreaks of foodborne illness due to *B. cereus* have been associated with the consumption of raw produce, including beans, cress, and mustard bean sprouts (Burnett and Beuchat, 2001). In particular, perishable agricultural products subject to short time frames for distribution and consumption can be contaminated with pathogenic *Bacillus* species, resulting in potential foodborne illness for consumers. Although outbreaks in the United States are considered under-reported, approximately 1% of bacterial outbreaks in the United States in 2015 were confirmed to be caused by

***Corresponding author:** Hyunjeong Cho, Moon S. Kim
Tel: +82-54-429-7765, +1-301-504-8462;
Fax: +82-54-429-7779, +1-301-504-9466
E-mail: hjcho201@korea.kr, Moon.Kim@ars.usda.gov

Copyright ⓒ 2018 by The Korean Society for Agricultural Machinery
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Bacillus (CDC, 2017). In Korea, outbreaks caused by *B. cereus* accounted for 2.1% of all food poisoning cases in 2017 (MFDS, 2018).

Conventional methods such as selective media culturing and polymerase chain reaction (PCR) are used to detect bacterial contamination in food. However, these methods are time-consuming and labor intensive. It is difficult to distinguish *B. thuringiensis* from *B. cereus* by using PCR because substances in the sample matrix often inhibit the PCR reaction, and the tests are sensitive to experimental conditions (Pahlow et al., 2015). Therefore, a rapid and reproducible method for detecting and distinguishing *B. cereus* and *B. thuringiensis* is necessary.

Raman spectroscopy provides structural information by detecting molecular vibrations caused by the inelastic scattering of laser light interacting with the sample. Raman shift coincides with the vibrational energies of the molecule. Vibrational spectroscopy can provide rapid and accurate results along with surface-enhanced Raman spectroscopy (SERS) technology, which enhances Raman scattering by approximately 10^3–10^6 folds, despite being an early step in bacterial identification (Sundaram et al., 2013). SERS substrates are utilized as either metallic nanoparticles or metallic surfaces in colloidal solutions (Schmidt et al., 2012). To achieve high sensitivity and low fluorescence interference, nanoparticles and substrates are generally used to detect species of bacteria (Kahraman et al., 2008; Premasiri et al., 2016). Nanoparticles are used as both antibacterial agents and SERS enhancers (Mosier-Boss, 2017), and gold and silver nanoparticles are applied as SERS enhancers to detect bacteria *E. coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* (Zhang et al., 2015; Yang et al., 2016a). However, only a few nanoparticle methods to detect *B. cereus* and *B. thuringiensis* have been reported. This study developed an easy-to-use, practical SERS method that was evaluated using five types of nanoparticles in colloidal solution to detect *B. cereus* and *B. thuringiensis*.

Materials and Methods

Bacterial materials

Colonies of *B. cereus* (*Bc*, ATCC 13061) and *B. thuringiensis* (*Bt*, USDA 708) were grown in Nutrient Agar (BBL™, BD, Franklin Lakes, NJ, USA) at 37°C for 24 h. Bacteria were collected using a sterile loop (1 μL) and then suspended in 3 mL of phosphate buffered saline (PBS, 1X). The McFarland turbidity was measured using a densitometer (DEN-1B, Grant Instruments, Cambridge, UK).

Nanoparticles and preparation

To obtain Raman spectra, five commercial nanoparticles were used. Four gold nanoparticles (AuNP) and one silver nanoparticle (AgNP) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and nanoComposix (San Diego, CA, USA), respectively. Their selection is based on their different stabilizing solvents, sizes of nanoparticles, and attached/unattached silica shells by searching for commercially available nanoparticles. In this paper, five nanoparticles are labeled as A, B, C, D, and E. Gold nanoparticle A (Sigma-Aldrich, 741965) of diameter 20 nm without silica was stabilized in citrate buffer. Gold nanoparticle B (Sigma-Aldrich, 753610) of diameter 20 nm without silica was stabilized in PBS buffer. Gold nanoparticle C (nanoComposix, AUSH50) of diameter 50 nm with silica coating (core diameter: 50 ± 4 nm and silica shell thickness: 20 ± 5 nm) was stabilized in ethanol. Gold nanoparticle D (nanoComposix, AUSH100) of diameter 100 nm with silica coating (core diameter: 100 ± 5 nm and silica shell thickness: 20 ± 5 nm) was stabilized in ethanol. Silver nanoparticle E (Sigma-Aldrich, 730793) of diameter 20 nm without silica was stabilized in an aqueous buffer containing sodium citrate. A volume of 30 μL of *Bacillus* species suspension was prepared per 30 μL of each nanoparticle to evaluate the utility of the nanoparticles. After vortexing, 30 μL of each mixture was placed on a nickel plate for Raman measurement.

Raman instrument and data analysis

Bacterial spectra were acquired using a 785-nm point-scan Raman system developed by scientists at the U.S. Department of Agriculture, Agricultural Research Service (Qin et al., 2010). Figure 1 shows the layout of the Raman system that includes a 785 nm laser module (I0785MM0500MF, Innovative Photonics Solutions, Monmouth Junction, NJ, USA). A 16 bit charge-coupled device (CCD) camera (Newton DU920N-BR-DD, Andor Technology, South Windsor, CT, USA) that can capture 1024 × 256 pixels and a Raman imaging spectrometer (Raman Explorer 785, Headwall Photonics, Fitchburg, MA, USA) are included in the point-scan Raman system.

The output power of a laser light source of wavelength 785 nm can be adjusted to a maximum of 500 mW. A bifurcated fiber optic probe (RBP, InPhotonics, Norwood, MA, USA) is used to focus the laser light on the sample and acquire the resulting Raman scattering signal. The stepping motor controller controls the movement of a two-axis motorized positioning table (MAXY4009W1-S4, Velmex, Bloomfield, NY, USA). This table moves the samples and is used to scan each sample repeatedly four times. A bifurcated optical fiber bundle connects the laser module, the Raman probe, and the Raman imaging spectrometer. The spectrometer acquires measurements over a Raman wavelength range of 791.3 nm to 980.3 nm, or a Raman shift range of 102.2 cm^{-1} to 2538.1 cm^{-1}.

An interface software was developed using LabVIEW platform (National Instruments, Austin, TX, USA). Data analysis was performed using MATLAB version 7.13 (R2011b) (The MathWorks, Inc., Natick, MA, USA). The fluorescence background was corrected using adaptive iteratively reweighted penalized least squares (airPLS). Percent relative standard deviation (% RSD) and t-test were performed using Microsoft® Excel® 2016 to determine the reproducibility and significance of Raman intensities of *Bc* and *Bt*.

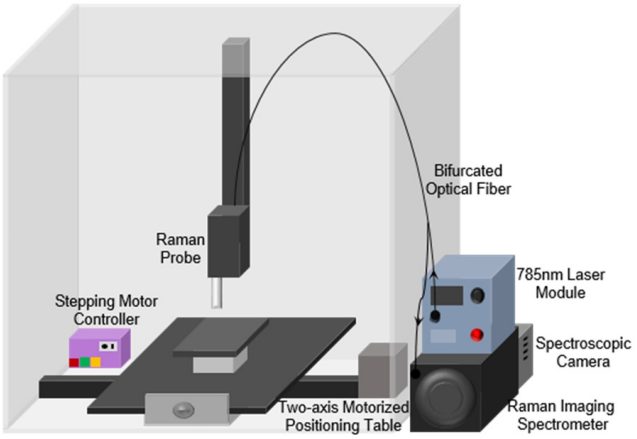

Figure 1. Layout of 785 nm point-scan Raman system.

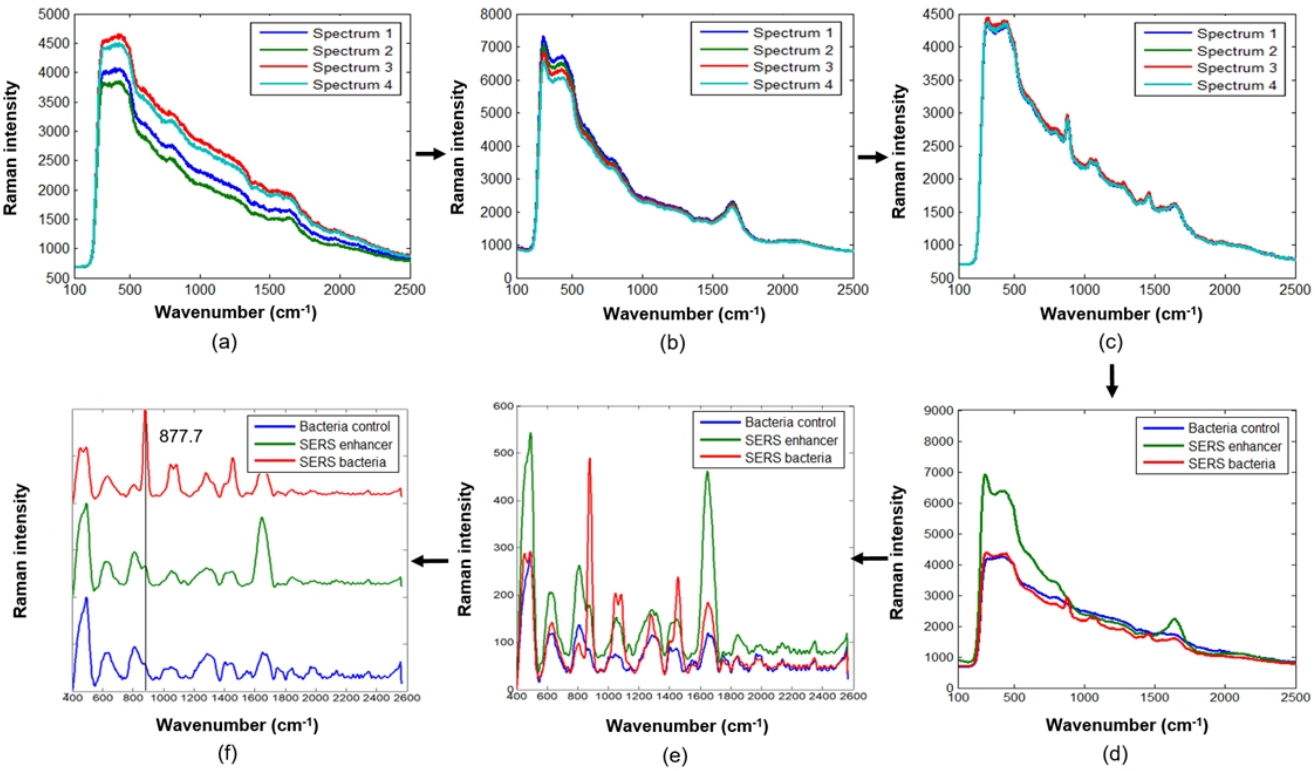

Figure 2. The procedure of Raman analysis to determine the SERS spectrum of *Bacillus thuringiensis* using gold nanoparticle A, stabilized in citrate buffer. (a) Original Raman spectra (bacteria control); (b) Original Raman spectra (SERS enhancer); (c) Original Raman spectra (SERS bacteria); (d) Mean Raman spectra; (e) Fluorescence corrected Raman spectra; (f) Normalized corrected Raman spectra. The SERS bacteria peaks are plotted in (f).

Results and Discussion

Raman spectra of *Bacillus cereus* and *Bacillus thuringiensis*

The suspended McFarland turbidity of both *Bc* and *Bt* was 3.5 that is equivalent to 1.1×10^9 colony forming units (CFU)/mL. Raman spectra for each *Bc* and *Bt* suspension without nanoparticles (bacteria control), for each of the five nanoparticle suspensions without bacteria (SERS enhancer), and for each pairwise mixture of bacterial suspension and nanoparticles (SERS bacteria) were obtained and analyzed. Figure 2 shows the stepwise

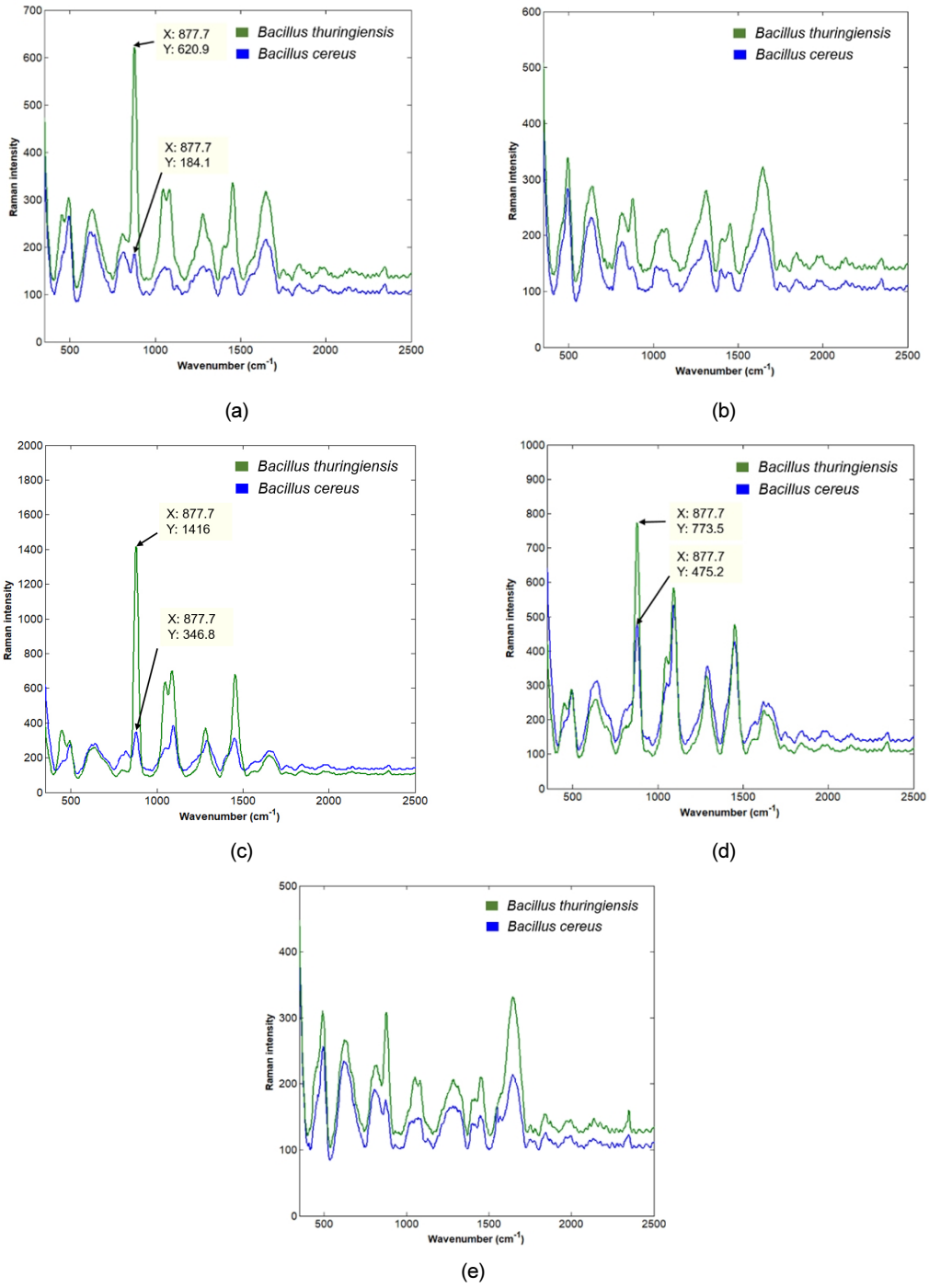

Figure 3. Raman intensities of five nanoparticles to detect *Bacillus cereus* and *Bacillus thuringiensis*. (a) The SERS bacteria peaks of nanoparticle A; (b) Peaks of nanoparticle B; (c) The SERS bacteria peaks of nanoparticle C; (d) The SERS bacteria peaks of nanoparticle D; (e) Peaks of nanoparticle E. The SERS bacteria peaks are labeled by black arrows in (a), (c) and (d).

process to obtain the SERS spectrum of *Bt* with gold nanoparticle A. After obtaining four spectra of each sample, the mean Raman spectrum and then the fluorescence-corrected Raman spectrum were calculated sequentially. The normalized spectra of bacteria control, SERS enhancer, and SERS bacteria could then be compared to determine if any SERS effect occurred for *Bt* and *Bc* in combination with the nanoparticle. Figure 2 shows that the highest intensity peak of the normalized SERS spectrum of *Bt* with nanoparticle A is at 877.7 cm^{-1}.

Evaluation of nanoparticles to detect *Bacillus cereus* and *Bacillus thuringiensis*

Figure 3 shows fluorescence corrected Raman spectra of *Bc* and *Bt* for five nanoparticles investigated in this study. With nanoparticle A, the Raman spectra of *Bc* and *Bt* did not show useful qualitative differences; however, the Raman intensities were quantitatively different, 184 and 620, respectively, with both peaks at 877.7 cm^{-1}. The peaks of *Bc* and *Bt* could not be distinguished using nanoparticle B. The difference between A and B gold nanoparticles is the type of buffer used to stabilize these nanoparticles. Nanoparticle A probably exhibits a higher SERS signal than nanoparticle B because the citrate ions of nanoparticle A generate negative charges on the surface of the nanoparticles (Zhou et al., 2012). During the interaction between bacterial cells and gold nanoparticle complexes (combination of gold nanoparticles and citric acid), Au nanoparticles are likely to attach to the bacterial cells, whereas the negatively charged citrate ions dissociate (Zhou et al., 2012). The enhanced Raman signal is therefore assumed to result from the attachment of Au nanoparticles to bacterial cells. The PBS buffer used for nanoparticle B is commonly used in bacterial samples and the effect of PBS buffer depends on its concentration and size (Wang et al., 2016). The commercial nanoparticles result in different Raman signals depending on the buffer composition used to stabilize the Au metal. We believe that most of the difference is due to the interaction between bacteria and nanoparticles stabilized in citrate *vs*. PBS buffer. *Bc* and *Bt* could not be distinguished qualitatively using nanoparticle C; however, the Raman intensities were remarkably different, 346 and 1,416, respectively, at 877.7 cm^{-1}. Nanoparticle C showed higher Raman intensity than nanoparticle A. Similar results have been observed by others, and are probably due to greater chemical stability and a higher permeability of core-shell nanoparticles to target cell, thereby providing a better signal (Khatami et al., 2018; Pang et al., 2016). The Raman intensities of *Bc* and *Bt* with nanoparticle D were 475 and 773 at 877.7 cm^{-1}, respectively, and spectral trends were similar to those exhibited by nanoparticles A and C. The only difference between nanoparticles C and D was particle size. The size of nanoparticle D was larger than the size of nanoparticle C. The core diameters of nanoparticles C and D were 50 ± 4 nm and 100 ± 4 nm, respectively, whereas their silica shell thicknesses were both 20 ± 5 nm. The size of nanoparticles probably influences the intensity of the Raman signal. It has been reported that the size of nanoparticles is crucial for Raman signal intensity, and it was observed that 15 nm AuNP showed a weaker signal than 50 nm AuNP (Yang et al., 2016b). The spectral profiles of *Bc* and *Bt* are very similar for all nanoparticles; however, subtle differences in peak intensity were observed in the Raman fingerprints. SERS signal was not observed in *Bc* and *Bt* with nanoparticle E, which is a silver nanoparticle. Gold and silver are commonly used as SERS substrates due to their stability in air (Sharma et al., 2012). Gold and silver nanoparticles showed different SERS activities in this study. It is assumed that gold and silver nanoparticles with different compositions and coatings would result in different SERS effects.

Subsequently, significant differences in Raman intensities between *Bc* and *Bt* were observed at 877.7 cm^{-1}

Table 1. Comparison of reproducibility and significance of Raman intensities between *Bacillus cereus* (*Bc*) and *Bacillus thuringiensis* (*Bt*)

No.	Nanoparticle A		Nanoparticle C		Nanoparticle D	
	Bc	*Bt*	*Bc*	*Bt*	*Bc*	*Bt*
1	190.81	628.16	342.97	1530.01	503.22	829.56
2	179.75	621.50	338.79	1385.97	466.11	756.30
3	196.44	636.70	384.22	1504.39	490.40	762.09
4	176.00	610.49	312.69	1329.82	443.24	723.67
Mean ± SD	185.75±9.50	624.21±11.06	344.67±25.61	1437.55±82.59	475.74±23.01	767.91±38.49
% RSD	5.12	1.77	7.43	5.75	4.84	5.01
t-test	0.00000 [a]		0.00003 [a]		0.00003 [a]	

[a] Statistically significant at the p-value of 0.05.

when using nanoparticles A, C, and D. To confirm the reproducibility and significance of the SERS spectra of *Bc* and *Bt*, the nanoparticles A, C, and D were selected and analyzed. The results are presented in Table 1 for % RSD and t-test analysis.

The % RSD of *Bc* and *Bt* are in the range of 4.84–7.43 and 1.77–5.75, respectively. The t-test for *Bc* and *Bt* is significant at the p-value of 0.05. For all three nanoparticles, *Bt* has greater Raman intensity than *Bc*. The gold nanoparticle stabilized in citrate buffer (nanoparticle A) has the lowest standard deviation for *Bc* and *Bt*, and exhibits high reproducibility. The gold nanoparticle stabilized in ethanol (nanoparticle D) shows a higher reproducibility than gold nanoparticle stabilized in ethanol (nanoparticle C) based on % RSD. The SERS spectra of *Bc* and *Bt* are very similar to those reported in previous studies (Kalasinsky et al., 2007); despite the different Raman shifts, the spectral profiles of *Bc* and *Bt* show similar SERS peak intensity differences in both current and previous studies, with the SERS signal of *Bt* showing a higher intensity than the *Bc* signal. It is assumed that *Bt* has parasporal protein crystals that affect the Raman signal. The SERS peak indicates interaction between the nanoparticle and the bacteria cell envelope that includes the cytoplasmic membrane and cell wall. The 877.7 cm^{-1} band is not reported to be associated with a specific component of the cell wall. These results can be further confirmed using application to produce such as leafy vegetables for the detection of *Bacillus* species. Further research may include the evaluation of substrates and SERS tags to improve the SERS signal.

Conclusions

Raman spectroscopy is a prospective alternative for the rapid detection of bacteria using SERS technology. In this study, the SERS effects of five types of nanoparticles were evaluated through the preparation of bacteria and nanoparticles, method development, and SERS measurements. This study evaluates commercially available nanoparticles for the demonstration of a simple and easy-to-use SERS method to detect *Bacillus*. The Raman intensities resulting from different nanoparticles vary. *B. cereus* and *B. thuringiensis* show subtle differences in the Raman intensities at 877.7 cm^{-1} when using three different gold nanoparticles. These results indicate that the bacteria cell wall interacts with nanoparticles and that combining bacteria with suitable nanoparticles will improve SERS signals, depending on the specific characteristics of each nanoparticle. We expect the results of this study to lay foundation for further research on practical SERS methods to detect bacteria, and furthermore, to differentiate species in the future, such as for pre-screening tools in fresh produce.

Conflict of Interest

The authors have no conflicting financial or other interests.

Acknowledgement

The authors would like to thank Patricia D. Miller of the Environmental Microbial and Food Safety Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture for providing bacteria samples. This research was partially supported by the cooperative agreement between the Experiment Research Institute, National Agricultural Products Quality Management Service, Korea, and the Agricultural Research Service, USDA, USA.

References

Ankolekar, C., T. Rahmati and R.G. Labbé. 2009. Detection of toxigenic *Bacillus cereus* and *Bacillus thuringiensis* spores in U.S. rice. International Journal of Food Microbiology 128(3): 460-466. http://doi.org/10.1016/j.ijfoodmicro.2008.10.006

Burnett, S.L. and L.R. Beuchat. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. Journal of Industrial Microbiology & Biotechnology 27(2): 104-110. http://doi.org/10.1038/sj.jim.7000199

Centers for Disease Control and Prevention (CDC). 2017. Surveillance for Foodborne Disease Outbreaks, United States, 2015, Annual Report. Atlanta, Georgia: US Department of Health and Human Services, CDC. Available at: www.cdc.gov (2018.7.18)

Kahraman, M., M.M. Yazıcı, F. Şahin and M. Çulha. 2008. Convective assembly of bacteria for surface- enhanced

Raman scattering. Langmuir 24(3): 894-901. http://doi.org/10.1021/la702240q

Kalasinsky, K.S., T. Hadfield, A.A. Shea, V.F. Kalasinsky, M.P. Nelson, J. Neiss, A.J. Drauch, G.S. Vanni and P.J. Treado. 2007. Raman chemical imaging spectroscopy reagentless detection and identification of pathogens: signature development and evaluation. Analytical Chemistry 79(7): 2658-2673. http://doi.org/10.1021/ac0700575

Khatami, M., H.Q. Alijani, M.S. Nejad and R.S. Varma. 2018. Core@shell nanoparticles: greener synthesis using natural plant products. Applied Sciences 8(3): 411. http://doi.org/10.3390/app8030411

Li, F., S. Zuo, P. Yu, B. Zhou, L. Wang, C. Liu, H. Wei and H. Xu. 2016. Distribution and expression of the enterotoxin genes of *Bacillus cereus* in food products from Jiangxi Province, China. Food Control 67: 155-162. http://doi.org/10.1016/j.foodcont.2016.02.049

Ministry of Food and Drug Safety (MFDS). 2018. Food poisoning outbreak DB. Available at: www.foodsafetykorea.go.kr (2018.7.18)

Mosier-Boss, P.A. 2017. Review on SERS of Bacteria. Biosensors 7(4): 51. http://doi.org/10.3390/bios7040051

Organji, S.R., H.H. Abulreesh, K. Elbanna, G.E.H. Osman and M. Khider. 2015. Occurrence and characterization of toxigenic *Bacillus cereus* in food and infant feces. Asian Pacific Journal of Tropical Biomedicine 5(7): 515-520. http://doi.org/10.1016/j.apjtb.2015.04.004

Pahlow, S., S. Meisel, D. Cialla-May, K. Weber, P. Rösch and J. Popp. 2015. Isolation and identification of bacteria by means of Raman spectroscopy. Advanced Drug Delivery Reviews 89: 105-120. http://doi.org/10.1016/j.addr.2015.04.006

Pang, S., T. Yang and L. He. 2016. Review of surface enhanced Raman spectroscopic (SERS) detection of synthetic chemical pesticides. Trends in Analytical Chemistry 85: 73-82. http://doi.org/10.1016/j.trac.2016.06.017

Premasiri, W.R., J.C. Lee, A. Sauer-Budge, R. Théberge, C.E. Costello and L.D. Ziegler. 2016. The biochemical origins of the surface-enhanced Raman spectra of bacteria: a metabolomics profiling by SERS. Analytical and Bioanalytical Chemistry 408(17): 4631-4647. http://doi.org/10.1007/s00216-016-9540-x

Qin, J., K. Chao and M.S. Kim. 2010. Raman chemical imaging system for food safety and quality inspection. Transactions of the ASABE 53(6): 1873-1882. http://doi.org/10.13031/2013.35796

Schmidt, M.S., J. Hübner and A. Boisen. 2012. Large area fabrication of leaning silicon nanopillars for surface enhanced Raman spectroscopy. Advanced Optical Materials 24(10): OP11-OP18. http://doi.org/10.1002/adma.201103496

Sharma, B., R.R. Frontiera, A.-I. Henry, E. Ringe and R.P. Van Duyne. 2012. SERS: materials, applications, and the future. Materials Today 15(1-2): 16-25. http://doi.org/10.1016/S1369-7021(12)70017-2

Sundaram, J., B. Park, A.H. Jr., K.C. Lawrence and Y. Kwon. 2013. Detection and differentiation of *Salmonella* serotypes using surface enhanced Raman scattering (SERS) technique. Food Measure 7(1): 1-12. http://doi.org/10.1007/s11694-012-9133-0

Vilas-Bôas, G.T., A.P.S. Peruca and O.M.N. Arantes. 2007. Biology and taxonomy of *Bacillus cereus, Bacillus anthracis, and Bacillus thuringiensis*. Canadian Journal of Microbiology 53(6): 673-687. http://doi.org/10.1139/W07-029

Wang, P., S. Pang, J. Chen, L. McLandsborough, S.R. Nugen, M. Fan and L. He. 2016. Label-free mapping of single bacterial cells using surface-enhanced Raman spectroscopy. Analyst 141(4): 1356-1362. http://doi.org/10.1039/C5AN02175H

Yang, D., H. Zhou, C. Haisch, R. Niessner and Y. Ying. 2016a. Reproducible *E. coli* detection based on label-free SERS and mapping. Talanta 146: 457-463. http://doi.org/10.1016/j.talanta.2015.09.006

Yang, T., Z. Zhang, B. Zhao, R. Hou, A. Kinchla, J.M. Clark and L. He. 2016b. Real-time and *in situ* monitoring of pesticide penetration in edible leaves by surface-enhanced Raman scattering mapping. Analytical Chemistry 88(10): 5243-5250. http://doi.org/10.1021/acs.analchem.6b00320

Zhang, H., X. Ma, Y. Liu, N. Duan, S. Wu and Z. Wang. 2015. Gold nanoparticles enhanced SERS aptasensor for the simultaneous detection of *Salmonella typhimurium* and *Staphylococcus aureus*. Biosensors and Bioelectronics 74: 872-877. http://doi.org/10.1016/j.bios.2015.07.033

Zhou, Y., Y. Kong, S. Kundu, J.D. Cirillo and H. Liang. 2012. Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *Bacillus* Calmette-Guérin. Journal of Nanobiotechnology 10: 19. http://doi.org/10.1186/1477-3155-10-19