ISSN 1392 – 1231. CHEMINĖ TECHNOLOGIJA. 2012. Nr. 3 (61)

Identification of different *Listeria monocytogenes* strains by surface enhanced FT Raman spectroscopy

K. Kairyte, Z. Luksiene
[1] *Vilnius University Institute of Applied Research*
Sauletekio Ave. 10, LT-10222 Vilnius, Lithuania
E-mail: Kristina.Kairyte@tmi.vu.lt

V. Sablinskas
Vilnius University Faculty of Physics
Sauletekio Ave. 9, LT-10222 Vilnius, Lithuania

crossref http://dx.doi.org/10.5755/j01.ct.61.3.2712

Received 17 September 2012; Accepted 24 October 2012

Recently developed genetic methods, compared to the traditional morphological and biochemical tests, have been designated as the "gold standard" for bacterial identification. However, these methods are time-consuming and require expensive reagents and expendables. In this context, Raman spectroscopy is gaining increasing attention. Our study demonstrates FT Raman spectra of thermotolerant and thermoresistant *Listeria monocytogenes* species, applying silver nanoparticles for enhancing the Raman signal. The region 600–800 cm^{-1} of SERS spectra of pathogenic bacteria is best suited for the identification and discrimination of these two pathogenic bacteria.

Key words: Raman spectroscopy, *Listeria*, silver colloid, bacteria identification

Introduction

Food-borne diseases are still an essential and significant problem around the world. More than 1.5 billion cases of food-borne illnesses and more than 3 million deaths from these diseases are estimated every year [1]. One of the main food pathogens, *Listeria monocytogenes*, causes gastroenteritis, but in immunocompromised individuals it can cause meningitis with a high mortality rate, and in pregnant women it can lead to abortion [2]. It is commonly found in soil, surface water, plants and foods, but also it finds favourable growth conditions on floors, drains and equipment within food industry premises, notably in the cold and wet atmosphere of refrigerated rooms where non-psychrotrophic bacteria can only survive [3].

In order to identify contaminated products and to safeguard public health, it is very important to have a quick and reliable method allowing to detect and identify *L. monocytogenes*. The recently developed genetic methods, namely analysis of 16S ribosomal deoxyribonucleic acid (DNA) or 16S ribosomal ribonucleic acid (RNA), have been designated as the "gold standard" for bacterial identification. However, these genetic methods have some drawbacks. First of all, they are time-consuming. Second, they require expensive reagents and expendables.

The spectroscopic techniques that detect molecular vibrations have been used for the analysis and identification of microorganisms over the last years. Infrared spectroscopy and Raman spectroscopy provide complementary technologies for a rapid and precise detection of microorganisms and are emerging methods in food analysis. These techniques are especially useful for studying the properties of bacterial biofilms on contact surfaces, the presence and viability of bacterial vegetative cells and spores, the type and degree of bacterial injury, and for the assessment of antibiotic susceptibility [4]. Infrared and Raman spectroscopies have been extensively applied in various research areas: for detection of food toxicants and chemical adulteration [5], bioprocessing and fermentation monitoring [6], enzyme activity [7], microorganism identification [8, 9]. Most of the latter studies were focused on the identification of different bacterial species. In this study, we have examined whether it is possible to distinguish bacterial strains. For this purpose, two different strains of the main food pathogen *Listeria monocytogenes* were chosen.

In order to have a reasonable signal-to-noise ratio, the surface-enhanced Raman spectra (SERS) in the latter studies were exited using a high (1 W) Nd:YAG laser power. Such power cannot guarantee the undamaged chemical structure of biological objects. Therefore, SERS experiments with low-power NIR excitation are needed for a proper assignment of SERS spectral bands of bacterial pathogens.

The aim of this study was focused on the possibility to identify thermotolerant and thermoresistant *Listeria monocytogenes* strains by surface-enhanced FT Raman spectroscopy, exciting samples by a low-power near-infrared laser.

Materials and methods

Bacterial culture and silver colloid preparation

Two food pathogens were used for experiments: the thermotolerant *Listeria monocytogenes* $ATC_{L3}C$ 7644 (3rd

passage of ATCC7644-test organism) was kindly provided by the National Veterinary Laboratory (Vilnius, Lithuania), and the thermoresistant *Listeria monocytogenes* 56Ly was kindly provided by UNIBO (Prof. E. Guerzoni, Italy).

All bacteria were maintained at 37 °C for 24 hours onto Luria Bertani Agar (LBA; Liofilchem, Roseto degli Abruzzi, Italy). Later, *Listeria* cultures were grown overnight (~16 h) at 37 °C in 20 ml of Tryptone Soya medium supplemented with 0.6 % Yeast Extract (TSYE) (Liofilchem). Afterwards, the bacterial cultures were 20 times diluted with the fresh TSYE medium (OD_{540} = 0.164) and grown at 37 °C in a shaker to the mid-log phase. Cells were then harvested by centrifugation (10 min, 3420×g), washed twice with sterile water and finally resuspended in a 0,1 ml of sterile water to the final concentration of about $1{,}5\times10^{10}$ CFU/ml. Silver colloids were prepared as described by a modified method of Lee and Meisel [10].

Briefly, distilled water (50 ml) was heated in a 100 ml flask with vigorous stirring on a Corning stirrer/hot plate (Model PC-620, Fisher Scientific Ltd., Ottawa, Ontario, Canada). At 45 °C, 9 mg of $AgNO_3$ (Sigma, St. Louis, MO) was added. This solution was heated until boiling (100 °C). Then, a 1 ml aliquot of 1 % (w/v) trisodium citrate (Sigma) was added into the solution and boiling was maintained for 60 min. The flask was covered with aluminium foil to minimise the evaporation loss of water. The obtained silver colloids were stored at 4 °C until use. The resulting nanoparticle suspensions had an average particle size of 40 nm.

SERS spectra acquisition

For SERS studies, a 6 µl aliquot of the bacterial suspension was mixed with a 6 µl aliquot of silver colloids that had been preagregated with NaCl in a 2 : 1 ratio (v/v) of colloids to NaCl (0.05 M). The samples were prepared on a conventional gold-plated mirror commonly used in FT-Raman spectrometer in a backscattering configuration.

The SERS spectra were obtained using the FT-Raman spectrometer MULTIRAM from Bruker. In order to avoid fluorescence background, the Raman experiments were performed in the near-infrared spectral region, using the Nd:YAG laser (λ = 1064 nm) as the excitation source. The laser operates at 1064 nm with a variable power (from 1 to 1000 mW) which can be changed with a 1 mW step. The ultrahigh sensitivity liquid-nitrogen-cooled Ge detector was used for capturing the Raman signal. An Au flat mirror was used as a plate for placing the samples. The plate was attached to a motorised *x*, *y*, *z* stage. The sampling area was monitored by a video camera. The SERS spectra were recorded in backscattering geometry. In order to increase the signal-to-noise ratio of the spectra, 1000 scans were summarized and averaged for each spectrum. The laser power was set at 150 mW. Such power reasoned only a small heating of the samples.

Results and discussion

It should be noted that the concentration of bacterial cells used in this experiment (1×10^8–5×10^8 cells in cm^2) produces no or an extremely weak conventional Raman spectrum (data not shown). In order to have a sufficient enhancement factor of the SERS spectra, the silver colloid particles have to be much smaller than the wavelength of laser radiation used for the excitation of the SERS spectra. In our experiments, the laser wavelength was much larger than the size of the colloid particles (100–200 nm); this ensured a sufficient enhancement of the Raman signal. The size of colloid nanoparticles was monitored with an atomic force microscope (AFM). The AFM image of the colloid is presented in Fig. 1. A thorough examination of the image shows that the average diameter of the nanoparticles of this colloid is 35–40 nm, while the thickness is only 3 nm.

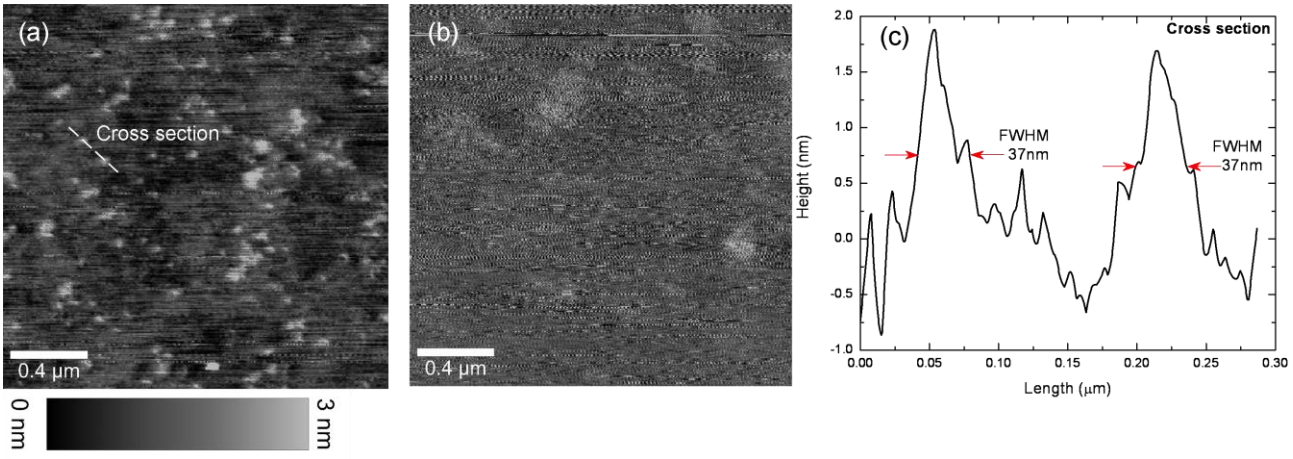

Fig. 1. (*a*) AFM image of silver colloid nanoparticles; (*b*) AFM image of glass substrate used for placing a drop of the colloid; (*c*) diagram for measuring the diameter and thickness of nanoparticles. The bar at the bottom of the figure shows a correlation between the thickness of a nanoparticle and the intensity of its grey colour in the image

The SERS spectra of thermotolerant and thermoresistant *Listeria monocytogenes* in the region 400–2000 cm^{-1} are presented in Fig. 2. The spectra consist of many spectral bands. The broad spectral feature in the

region 2400–3400 cm^{-1} is related to the thermal radiation of the sample; therefore, it was subtracted from the spectra. The intensity of this band correlates with the Nd:YAG laser power. Moreover, the spectra contain some additional Raman bands which can be attributed to the colloid. These bands can be easily subtracted from the spectra of the *L. monocytogenes* cells.

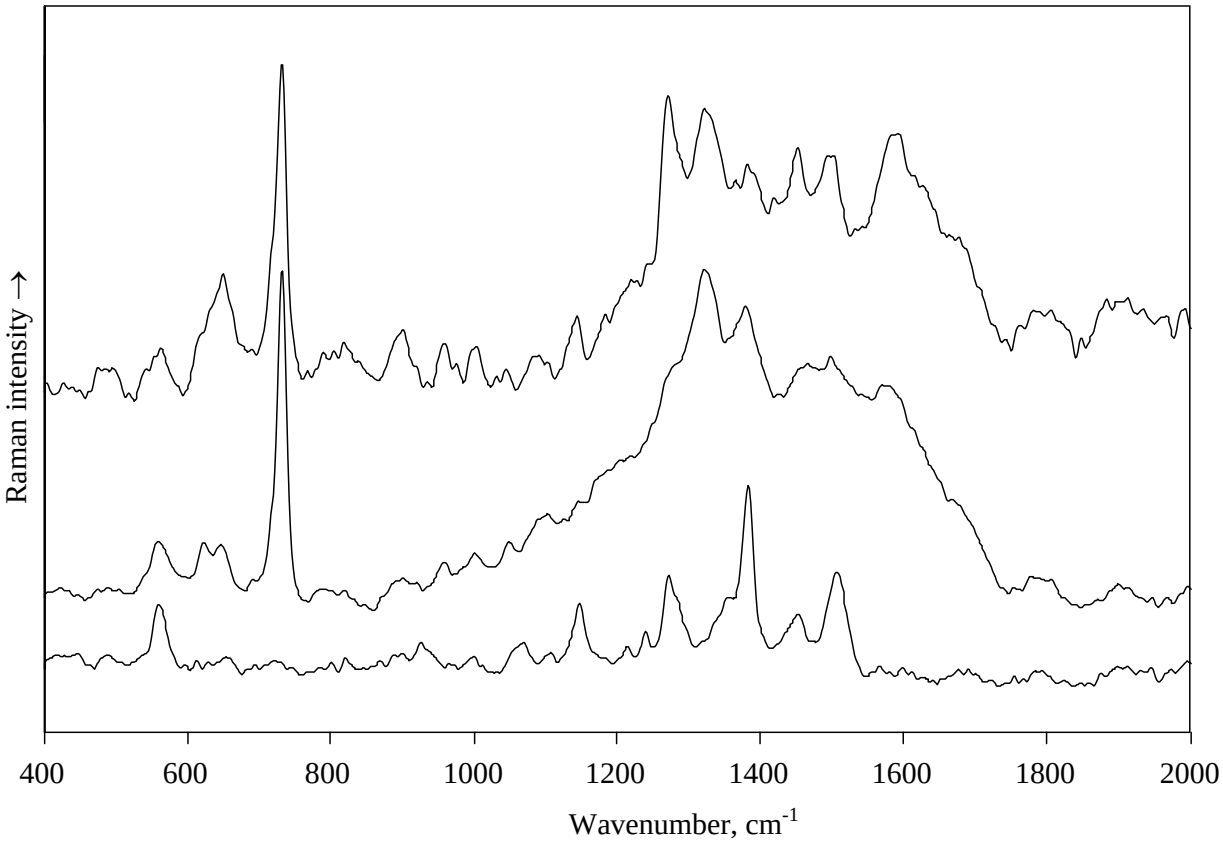

Fig. 2. SERS with NIR excitation (1064 nm) spectra of thermotolerant (top) and thermoresistant (middle) *Listeria monocytogenes* in the spectral region of 400–2000 cm^{-1}. The bottom curve represents the Raman spectrum of the silver colloid

An exact assignment of spectral bands of such chemically complicated system is not possible, but a tentative interpretation can be performed. The bands can be attributed to the main constituents of DNA and proteins, for instance, guanine, adenine, phenylaniline, cysteine, tryptophan and other amino acids, which are known to have large enhancement factors in SERS spectra. The most intense spectral bands can be attributed [11]: 621 cm^{-1} to phenylalanine, 646 cm^{-1} to the COO– band of amino acids or C–S stretch mode of cysteine, 732 cm^{-1} to glycosydic ring/adenine/CH_2 rocking, 956 cm^{-1} to N–C stretching, 1030 cm^{-1} to phenylalanine, 1091 cm^{-1} to C–C skeletal and COC stretching from the glycosydic link, 1325 cm^{-1} to CH_2 deformation, and 1586 cm^{-1} to the ring stretching of guanine and adenine.

Despite the similarities between SERS spectra of the bacteria, some spectral features can be used for the identification of these bacteria. The spectral differences for thermotolerant and thermoresistant *Listeria monocytogenes* are most prominent in the region 600–800 cm^{-1}, where spectral bands of skeletal vibrations of phenylalanine and cysteine are located. According to the intensities of the SERS spectral bands, the cysteine/phenylalanine ratio in the thermotolerant *Listeria* is higher than in the thermoresistant *Listeria*. This is not surprising, considering the differences between the chemical constitutions of the cell walls of both bacteria.

A comparison of bacterial SERS measurements presented in this study with data of other studies reported in the literature is difficult due to a large variety of results obtained in different experimental conditions (various SERS substrates, preparation protocols for the colloids and bacteria, different species and strains of bacteria, excitation wavelengths, etc.). Nevertheless, the obtained data are in good agreement with those published by Luo and Lin [11]. These authors have revealed several unique SERS peaks of *L. monocytogenes* at 627, 732, 957, 1097, 1331 and 1458 cm^{-1} with the presence of a predominant peak at 732 cm^{-1}.

It is worth noting that small molecular differences between thermotolerant and thermoresistant *Listeria*, which are difficult to identify biochemically, can be ascertained from SERS spectral differences. Moreover, some spectral regions (600–800 cm^{-1}) can be considered as "fingerprints" of bacterial cells, as they represent the overall molecular constituents of the selected bacteria.

Conclusions

The Nd:YAG laser (λ = 1064 nm) was used for the excitation of surface-enhanced Raman spectra of two different *Listeria* strains, applying silver nanoparticles for the enhancement of the Raman signal. The region 600–800 cm^{-1} of SERS spectra of pathogenic bacteria is best suited to identify and differentiate these two pathogenic bacteria.

References

1. **World Health Organization.** Food Safety and Food-Borne Illness // 2007. <http://www.who.int/mediacentre/factsheets/fs237/en/> (last accessed 11.09.23).
2. **Cossart P., Archambaud C.** The bacterial pathogen *Listeria monocytogenes*: an emerging model in prokaryotic transcriptomics // Journal of Biology. 2009. Vol. 8. P. 8:107.1–107.4.
3. **Carpentier B., Cerf O.** Review — Persistence of Listeria monocytogenes in food industry equipment and premises // International Journal of Food Microbiology. 2011. Vol. 145. P. 1–8. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.01.005
4. **Lu X., Al-Qadiri H. M., Lin M., Rasco B. A.** Application of mid-infrared and Raman spectroscopy to the study of bacteria // Food and Bioprocess Technology. 2011. Vol. 4. P. 919–935. http://dx.doi.org/10.1007/s11947-011-0516-8
5. **Cheung W., Shadi I. T., Xu Y., Goodacre R.** Quantitative analysis of the banned food dye Sudan-1 using surface enhanced Raman scattering with multivariate chemometrics // Journal of Physical Chemistry C. 2010. Vol. 114, N 16. P. 7285–7290. http://dx.doi.org/10.1021/jp908892n
6. **Clarke S. J., Littleford R. E., Smith W. E., Goodacre R.** Rapid monitoring of antibiotics using Raman and surface enhanced Raman spectroscopy // The Analyst. 2005. Vol. 130. N 7. P. 1019–1026. http://dx.doi.org/10.1039/b502540k
7. **Hollywood K. A., Shadi I. T., Goodacre R.** Monitoring the succinate dehydrogenase activity isolated from mitochondria by surface enhanced Raman scattering // Journal of Physical Chemistry C. 2010. Vol. 114. N 16. P. 7308–7313. http://dx.doi.org/10.1021/jp908950x
8. **Huang W. E., Li M., Jarvis R. M., Goodacre R., Banwart S. A.** Shining light on the microbial world: the application of Raman microscopy // Advances in Applied Microbiology. 2010. Vol. 70. P. 153–186. http://dx.doi.org/10.1016/S0065-2164(10)70005-8
9. **Davis R., Irudayaraj J., Reuhs B. L., Mauer L. J.** Detection of *E. coli* O157:H7 from ground beef using Fourier transform infrared (FT-IR) spectroscopy and chemometrics // Journal of Food Science. 2010. Vol. 75. N 6. P. 340–346. http://dx.doi.org/10.1111/j.1750-3841.2010.01686.x
10. **Lee P. C., Meisel D.** Adsorption and surface-enhanced Raman of dyes on silver and gold sols // Journal of Physical Chemistry. 1982. Vol. 86. P. 3391–3395. http://dx.doi.org/10.1021/j100214a025
11. **Luo B. S., Lin M.** A portable Raman system for the identification of foodborne pathogenic bacteria // Journal of Rapid Methods & Automation in Microbiology. 2008. Vol. 16. N 3. P. 238–255. http://dx.doi.org/10.1111/j.1745-4581.2008.00131.x

K. Kairytė, Ž. Lukšienė, V. Šablinskas

PAVIRŠIUMI SUSTIPRINTOS FT RAMANO SPEKTROSKOPIJOS TAIKYMAS SKIRTINGŲ *LISTERIA MONOCYTOGENES* BAKTERIJŲ PADERMĖMS APTIKTI

S a n t r a u k a

Šiuo metu bakterijoms rasti taikomi šiuolaikiniai genetiniai metodai, pranašesni už tradicinius morfologinius ir biocheminius testus. Tačiau ir genetiniai metodai turi trūkumų – yra brangūs, ilgesnė jų trukmė. Todėl ieškoma naujų, tikslių, greitų ir nebrangių metodų, kuriais būtų galima aptikti ir diferencijuoti patogeninius mikroorganizmus. Darbe dviem *Listeria monocytogenes* bakterijoms rasti pritaikyta Ramano spektroskopija. Signalui stiprinti panaudotas sidabro nanodalelių koloidinis tirpalas. Iš darbo rezultatų galima daryti išvadą, kad termostabilių ir termorezistentiškų *Listeria monocytogenes* bakterijų padermėms aptikti ir diferencijuoti šiuo spektriniu metodu tinkamiausia spektrinių juostų sritis yra 600–800 cm^{-1}.