Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2015 August ; 9550: . doi:10.1117/12.2188647.

Using Raman Spectroscopy and SERS for in-situ studies of rhizosphere bacteria

Sneha Poliseti^{*1}, Nameera Baig², Amber Bible³, Jennifer Morrell-Falvey³, Mitchel Doktycz³, Paul W. Bohn^{1,2}

¹Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556

²Department of Chemistry, University of Notre Dame, Notre Dame, IN 46556

³BioSciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

Abstract

Bacteria colonize plant roots to form a symbiotic relationship with the plant and can play an important role in promoting plant growth. Raman spectroscopy is a useful technique to study these bacterial systems and the chemical signals they utilize to interact with the plant. We present a Raman study of *Pantoea* YR343 that was isolated from the rhizosphere of *Populus deltoides* (Eastern Cottonwood). *Pantoea* sp. YR343 produce yellowish carotenoid pigment that play a role in protection against UV radiation, in the anti-oxidative pathways and in membrane fluidity. Raman spectroscopy is used to non-invasively characterize the membrane bound carotenoids. The spectra collected from a mutant strain created by knocking out the crtB gene that encodes a phytoene synthase responsible for early stage of carotenoid biosynthesis, lack the carotenoid peaks. Surface Enhanced Raman Spectroscopy is being employed to detect the plant phytohormone indoleacetic acid that is synthesized by the bacteria. This work describes our recent progress towards utilizing Raman spectroscopy as a label free, non-destructive method of studying plant-bacteria interactions in the rhizosphere.

Keywords

Raman spectroscopy; bacteria; SERS; auxins; pyocyanin

1. INTRODUCTION

Vibrational Raman spectroscopy is being increasingly recognized as a non-destructive method of studying biological samples close to their natural environment. Unlike IR spectroscopy, which is affected by the strong dipole moment of water and thus is restricted to dried samples, Raman can be applied to fresh biological samples with minimal sample preparation¹. This offers the advantage of examining live samples and carrying out time studies to observe changes in chemical signatures. Surface enhanced Raman spectroscopy (SERS) utilizes roughened coinage metal surfaces like colloids or patterned substrates to

^{*} spoliset@nd.edu; phone 1 574 631-1835; bohnresearchgroup.com.

dramatically enhance the inherently weak Raman signal. Studies have shown its capability to detect single molecules².

There has been growing interest in understanding the behavior and functioning of bacterial populations through the secreted chemical signatures and metabolites^{3, 4, 5}. Bacterial communities play a vital role in health, food and the environment and due the intrinsic complexity of these systems, a lot remains to be understood about their function and interactions. The rhizosphere microbiome associated with the plant root is key for plant health and can affect plant growth directly or indirectly. Our focus has been on using Raman spectroscopy and imaging in conjunction with various enhancing protocols and multivariate statistics to study rhizosphere and medically relevant bacterial communities. In the current paper we describe recent progress towards in-situ studies of bacterial communities.

2. METHODS

Sample preparation:

All strains were plated on Luria-Bertani (LB) agar plates and the cultures were grown in FAB minimal medium with glucose as the carbon source. Samples for Raman spectroscopy were pipetted onto 1-inch square silicon wafers. Silver colloid used for SERS enhancement was prepared according to the standard protocol by Lee and Meisel, resulting in 10-14nm nanoparticles⁶. Chloroform extraction of pyocyanin from *P. aeruginosa* strain FRD1 cultures was carried out using 3ml of chloroform per 6ml of overnight culture. After vigorous shaking, 50 μ l of the blue organic layer was mixed with 100 μ l of the Ag colloid and dried on silicon wafers in the dark. Plant related strains (YR343 and crtB) were obtained from ORNL and *P. aeruginosa* strain FRD1 was obtained from Dr. Joshua Shrout's laboratory at the University of Notre Dame.

Instrumentation & data analysis:

A confocal Raman microscope (Alpha 300R, WiTec, GMBH, Germany) equipped with a 532nm laser was used for collecting Raman and SERS spectra and images. Data processing was done in IGOR Pro 6.36 and MATLAB R2012b.

3. RESULTS AND DISCUSSION

Carotenoids in *Pantoea* sp. YR343

Carotenoids are known to play a role in protecting cells against cell damage by short-wavelength visible and UV radiation⁷. Raman spectroscopy is a suitable technique to characterize the pigments in live cells⁸. *Pantoea* species YR343 was isolated from the rhizosphere of *Populus deltoides* or eastern cottonwood. The 532 nm excitation wavelength matches well with the electronic absorption spectrum to produce selective enhancement of certain bands. Figure 1 consists of the resonance Raman spectra of YR343 overlaid with the spectra of the mutant strain crtB from which the gene encoding phytoene synthase has been knocked out. In the orange YR343 spectra two strong bands appear at 1512-1520 cm^{-1} assigned to $\nu(\text{C}=\text{C})$ in phase stretching and 1150-1160 cm^{-1} assigned to $\nu(\text{C}-\text{C})$ stretching vibrations of the polyene chain and a band of medium intensity appears at 995-1005 cm^{-1}

due to in plane rocking mode of CH₃ groups attached to the polyene chain⁹. These are absent in the carotenoid mutant strain. YR343 contains a mixture of many carotenoids and further characterization is being carried out to identify them.

Auxin production in YR343

Microbial synthesis of plant phytohormone auxin not only plays a role in plant processes like growth but is also a signaling molecule in bacterial communities^{10, 11}.

We used a colorimetric assay known as the Salkowski test to confirm synthesis of auxin indoleacetic acid (IAA) by YR343. Cultures were grown using exogenously added tryptophan as a precursor for the auxin production. The cultures treated with the Salkowski reagent (2% 0.5M FeCl₃ in 35% perchloric acid) show a red color due to formation of an iron complex as shown in figure 2 with varying concentrations of added tryptophan. The challenge of carrying out Raman spectroscopy to detect the presence of IAA is the high degree of similarity in the Raman spectra of IAA and tryptophan as shown in figure 3. We are currently working on addressing this issue by using a combination of SERS and chemometrics.

SERS detection of pyocyanin in *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a gram-negative bacterium that is an opportunistic pathogen. It produces a variety of pigments including blue-green pyocyanin, a toxin and virulence factor. Pyocyanin is known to play a role in the persistence of *P. aeruginosa* in patients with cystic fibrosis and is often used as a biomarker to detect the presence of *P. aeruginosa* infections¹². We carried out chloroform extraction of overnight cultures of *P.aeruginosa* mucoid strain FRD1, isolated from the lungs of a cystic fibrosis patient. The Raman spectrum without the silver colloid was unintelligible because of the large amount of sample autofluorescence. SERS enabled obtaining a spectrum of pyocyanin that matched well with the standard as shown in figure 4. Current efforts are focused on in-situ detection of pyocyanin in *P. aeruginosa* cultures.

4. CONCLUSIONS

In this paper we have described our recent efforts in using Raman spectroscopy and SERS to study plant rhizosphere and health related bacterial communities. Raman spectrum of carotenoids was obtained from live cells without any sample pre-treatment which shows the potential of Raman to be used for studying these systems with minimum perturbation of the natural environment. Future directions include in-situ detection of metabolites in bacterial culture samples using SERS and multivariate statistics for data analysis.

ACKNOWLEDGMENTS

This work was supported by the DOE Office of Science grant PTX-UT-Battlle ORNL 4000132808 (S. P.) and National Institute of Health grant 1R01AI113219-01 (N. B.).

REFERENCES

- [1]. Sengupta A, Mujacic M and Davis EJ , "Detection of bacteria by surface-enhanced Raman spectroscopy," *Analytical and bioanalytical chemistry* 386(5), 1379–1386 (2006). [PubMed: 16933128]
- [2]. Kneipp K, Wang Y, Kneipp H, Perelman LT, Itzkan I, Dasari RR and Feld MS , "Single molecule detection using surface-enhanced Raman scattering (SERS)," *Phys.Rev.Lett* 78(9), 1667 (1997).
- [3]. Bauer WD and Mathesius U , "Plant responses to bacterial quorum sensing signals," *Curr.Opin.Plant Biol* 7(4), 429–433 (2004). [PubMed: 15231266]
- [4]. Parsek MR and Greenberg E , "Sociomicrobiology: the connections between quorum sensing and biofilms," *Trends Microbiol.* 13(1), 27–33 (2005). [PubMed: 15639629]
- [5]. Lanni EJ, Masyuko RN, Driscoll CM, Dunham SJ, Shrout JD, Bohn PW and Sweedler JV , "Correlated imaging with C60-SIMS and confocal Raman microscopy: visualization of cell-scale molecular distributions in bacterial biofilms," *Anal.Chem* 86(21), 10885–10891 (2014). [PubMed: 25268906]
- [6]. Lee P and Meisel D , "Adsorption and surface-enhanced Raman of dyes on silver and gold sols," *J.Phys.Chem* 86(17), 3391–3395 (1982).
- [7]. Kirschfeld K , "Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells," *Proc.R.Soc.Lond.B.Biol.Sci* 216(1202), 71–85 (1982). [PubMed: 6137825]
- [8]. Puppels G, De Mul F, Otto C, Greve J, Robert-Nicoud M, Arndt-Jovin D and Jovin T , "Studying single living cells and chromosomes by confocal Raman microspectroscopy," (1990).
- [9]. Robert B , "The electronic structure, stereochemistry and resonance Raman spectroscopy of carotenoids," , 189–201 (1999).
- [10]. Duca D, Lorv J, Patten CL, Rose D and Glick BR , "Indole-3-acetic acid in plant-microbe interactions," *Antonie Van Leeuwenhoek* 106(1), 85–125 (2014). [PubMed: 24445491]
- [11]. Spaepen S and Vanderleyden J , "Auxin and plant-microbe interactions," *Cold Spring Harb Perspect.Biol* 3(4), 10.1101/cshperspect.a001438 (2011)
- [12]. Usher LR, Lawson RA, Geary I, Taylor CJ, Bingle CD, Taylor GW and Whyte MK , "Induction of neutrophil apoptosis by the *Pseudomonas aeruginosa* exotoxin pyocyanin: a potential mechanism of persistent infection," *J.Immunol* 168(4), 1861–1868 (2002). [PubMed: 11823520]

Figure 1:
Resonance Raman spectra of *Pantoea* sp. YR343 and ΔcrtB using a 532nm wavelength laser

Figure 2:
YR343 culture samples treated with Salkowski reagent. Red color indicates presence of IAA. The first five samples from left to right contain varying amounts of tryptophan and the sample to the right is a tryptophan control which is colorless after addition of reagent.

Figure 3:
Raman spectra of IAA (upper) and tryptophan (lower) showing similarity of spectra

Figure 4: SERS spectrum of pyocyanin from *Pseudomonas aeruginosa* strain FRD1 (top) overlaid with SERS spectrum of pyocyanin standard (bottom)