

## Detection of p-coumaric acid from cell supernatant using surface enhanced Raman scattering

**Morelli, Lidia; Jendresen, Christian Bille; Zor, Kinga; Rindzevicius, Tomas; Schmidt, Michael Stenbæk; Nielsen, Alex Toftgaard; Boisen, Anja**

*Published in:*  
Procedia Technology

*Link to article, DOI:*  
[10.1016/j.protcy.2017.04.081](https://doi.org/10.1016/j.protcy.2017.04.081)

*Publication date:*  
2017

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Morelli, L., Jendresen, C. B., Zor, K., Rindzevicius, T., Schmidt, M. S., Nielsen, A. T., & Boisen, A. (2017). Detection of p-coumaric acid from cell supernatant using surface enhanced Raman scattering. *Procedia Technology*, 27, 190-192. DOI: 10.1016/j.protcy.2017.04.081

## DTU Library

Technical Information Center of Denmark

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Biosensors 2016

## Detection of p-coumaric acid from cell supernatant using surface enhanced Raman scattering

L. Morelli<sup>a\*</sup>, C. Bille Jendresen<sup>b</sup>, K. Zor<sup>a</sup>, T. Rindzevicius<sup>a</sup>, M. Stenbæk Schmidt<sup>a</sup>, A. Toftgaard Nielsen<sup>b</sup>, A. Boisen<sup>a</sup>

<sup>a</sup> Department of Micro- and Nanotechnology, Technical University of Denmark, 2800 Kgs. Lyngby, DENMARK

<sup>b</sup> The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, 2970 Hørsholm, DENMARK

---

### Abstract

A standard protocol for analysis of microbial factories requires the screening of several populations in order to find the best performing ones. Standard analytical methods usually include high performance liquid chromatography (HPLC), thin layer chromatography (TLC) or spectrophotometry, which are expensive and time-consuming processes. Surface Enhanced Raman Spectroscopy (SERS), instead, is a highly sensitive spectroscopic technique for specific, fast and real-time sensing of biological samples. Here we demonstrate the use of SERS to discriminate between two different bacterial populations based on detection of p-coumaric acid (pHCA) in cell supernatant.

SERS active substrates, based on leaning gold-capped silicon nanopillars, were used for detection. They were successfully used to detect culture medium spiked with pHCA, and the effect of medium dilution was studied. For analysis of biological production of pHCA, triplicate cultures of *E. coli* strains expressing a pHCA-forming enzyme (P) as well as of a non-producing strain (C) were grown. Then, supernatant samples were collected and their pHCA content was measured using SERS and HPLC for comparison. The intensity of the pHCA Raman mode at  $1169\text{ cm}^{-1}$  (CH-rocking motion) showed different trends for P and C strains, similar to the results obtained using the HPLC method. Results illustrate that SERS can be used for quick and semi-quantitative discrimination of pHCA concentrations in cell supernatant medium.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Peer-review under responsibility of the organizing committee of Biosensors 2016

**Keywords:** Surface enhanced Raman spectroscopy; p-coumaric acid; *E. coli*; microbial factories; fast analysis

---

---

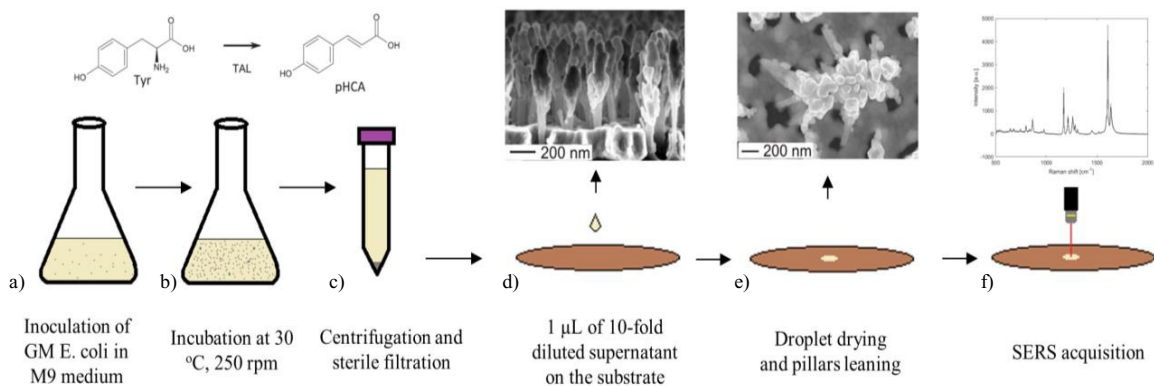
\* Corresponding author. Tel.: +4591734340.  
E-mail address: [lmor@nanotech.dtu.dk](mailto:lmor@nanotech.dtu.dk)

## 1. Introduction

Bacterial factories using genetically modified microorganisms as hosts, such as *E.coli* or *S.cerevisiae*, have proven efficient in the production of *p*-coumaric acid (pHCA) as a non-native secondary metabolite through non-oxidative deamination of tyrosine (Tyr) by tyrosine-ammonia lyase (TAL) [1,2]. As different modifications lead to different synthesis yields, a quantitative investigation is of crucial importance in order to find the best performing strain. Current standard methods include HPLC, TLC and spectrophotometry. These methods are sensitive and accurate, but they are time consuming and require large and expensive instrumentation. We suggest SERS, instead, as an alternative technique for quick analysis of biological samples. It is a molecule-specific, well-established spectroscopic technique which only requires drying a droplet of solution on an active substrate, with an analysis time of few minutes. SERS takes advantage of nanostructured metallic surfaces to enhance the weak Raman signal coming from analytes in solution. Our work is based on the SERS substrates developed by Schmidt et al. [3], based on flexible, metal-capped nanopillars, which can be produced at a wafer scale with high uniformity. Hence, we describe a simple and fast sampling procedure for qualitative screening of genetically modified *E.coli* strains, able to differentiate between a producing and a control strain within an analysis time of few minutes.

## 2. Results and discussion

Triplicate cultures of *E. coli* strains expressing TAL from *Flavobacterium johnsoniae* (and thus producing pHCA) (P) as well as control (C) strains were cultured in Tyr enriched medium according to the methods described in [1] (Fig. 1 (a)-(c)). Samples of cell supernatant were collected at 0, 3, 24 and 48 hours post seeding and their pHCA content was measured with HPLC, with the results shown in Fig. 1 (h). For SERS measurements, droplets of supernatant were dried on SERS active substrates, and acquired with a Raman microscope (Fig. 1 (d)-(f)). A high concentration of salts and other molecules in the sample matrix tend to form crystals on the active surface, thus preventing the leaning of nanopillars towards each other. The leaning effect of nanopillars (Fig. 1 (e), SEM image) creates regions called “hotspots”, where the enhancement of Raman signal takes place. Hence, the presence of salts significantly lowers the SERS signal coming from pHCA. For this reason, dilution of supernatant samples is needed. As shown in Fig. 1 (g), pHCA 100  $\mu$ M spiked in M9 medium diluted with MilliQ water at different ratios was analyzed. As the dilution ratio of the medium increases, the amplitude of the signal increases as well, as the clogging effect of the salts decreases. However, when diluting the supernatant, the concentration of pHCA decreases as well, leading to lower sensitivity. Therefore, 1:10 dilution was chosen as a compromise between matrix and pHCA dilution for supernatant analysis. A MatLab analysis was performed to extract the height of the significant peak at  $1169\text{ cm}^{-1}$ , with the results shown in Fig. 1 (i). The amplitude of peaks shows a similar trend compared to HPLC, being able to differentiate between P and C strains.



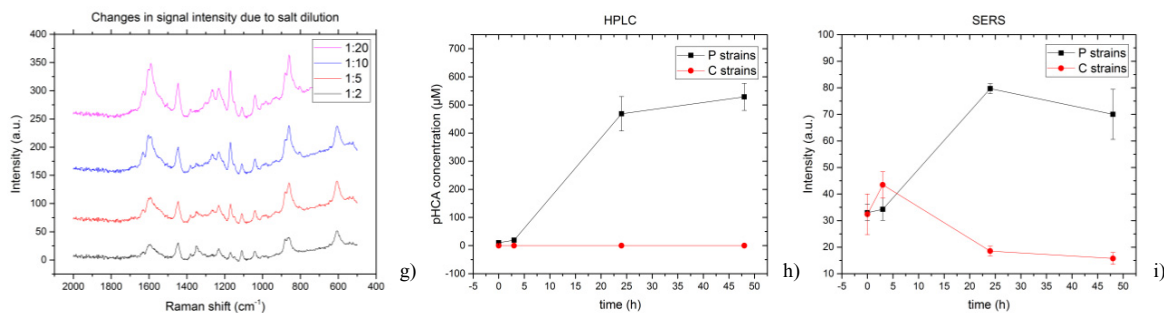


Fig. 1. Steps for bacterial culture and supernatant extraction: M9 culture medium with 1.8 mM Tyr is prepared, and aliquots of P and C strains of *E. coli* are inoculated (a) and incubated at 30 °C, 250 rpm in orbital shaker until the end of the experiment (b). During incubation, TAL converts Tyr into pHCA (insert). Aliquots of solution are taken from the cultures, centrifuged and sterile filtered at 0, 3, 24 and 48 h (c). 0.5 mL of supernatant are used for HPLC analysis, whereas 1 µL of 10-fold diluted supernatant is poured (d) and dried on the SERS substrate (e). SEM images (courtesy of Kaiyu Wu) show that the nanopillars stand vertically before wetting. After drying, surface tension makes the pillars lean close to each other, creating hotspots where the enhancement is higher. Finally a SERS map is acquired for each sample with a DXR Raman microscope (Thermo Fisher Scientific Inc.) at 780 nm, 1 mW, 10x objective, 25 µm slit (f). 100 µM pHCA spiked in culture medium diluted with MilliQ water in different ratios (1:2, 1:5, 1:10, 1:20) (g). Concentration of pHCA in cell supernatant for producing (P, black) and control (C, red) strains measured with HPLC (h). Each point is the average of 3 measurements, each one obtained from one of the triplicates. SERS signal at 1169 cm<sup>-1</sup> after baseline correction (i). Each point in the graph is the average of 3 maps of 25 points, whereas the error bars represent the standard error of the mean, calculated on the 3 average values.

### 3. Conclusions

In this work we demonstrated that SERS is a rapid and effective tool for qualitative screening of bacterial strains, based on the amount of synthesized secondary metabolites (e.g. pHCA). These results open up new possibilities for high-throughput quantitative analysis. Currently we are focusing on improving sensitivity by extracting pHCA in organic solvent and on integration of the assays on automated and high-throughput microfluidic platforms, such as lab-on-discs.

### References

- [1] C. B. Jendresen, S. G. Stahlhut, M. Li, P. Gaspar, S. Siedler, J. Förster, J. Maury, I. Borodina, and A. T. Nielsen, "Novel highly active and specific tyrosine ammonia-lyases from diverse origins enable enhanced production of aromatic compounds in bacteria and yeast," *Appl. Environ. Microbiol.*, vol. 81, no. 13, pp. AEM.00405–15, 2015.
- [2] T. Vannelli, W. Wei Qi, J. Sweigard, A. a. Gatenby, and F. S. Sariaslani, "Production of p-hydroxycinnamic acid from glucose in *Saccharomyces cerevisiae* and *Escherichia coli* by expression of heterologous genes from plants and fungi," *Metab. Eng.*, vol. 9, no. 2, pp. 142–151, 2007.
- [3] M. S. Schmidt, J. Hübner, and A. Boisen, "Large area fabrication of leaning silicon nanopillars for Surface Enhanced Raman Spectroscopy," *Adv. Mater.*, vol. 24, no. 10, pp. 11–18, 2012.