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Detection of molecular changes induced by antibiotics in *Escherichia coli* using vibrational spectroscopy

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ABSTRACT:

This study aimed to test Raman(400-1800 cm^{-1}) and Infra-red (1900-500 cm^{-1}) spectroscopies followed by statistical analysis (principal component analysis) to detect molecular changes induced by antibiotics (ampicillin, cefotaxime – cell wall synthesis inhibitors, tetracycline – protein synthesis inhibitor, ciprofloxacin – DNA synthesis inhibitor) against*Escherichia coli* TOP10. In case of ampicillin and cefotaxime, a decrease in protein bands in both Raman (1240, 1660 cm^{-1}), and IR spectra (1230, 1530, 1630 cm^{-1}), and an increase in carbohydrate bands (1150, 1020 cm^{-1}) in IR spectra were observed. Tetracycline addition caused an increase in nucleic acid bands (775, 1478, 1578 cm^{-1}), a sharp decrease in phenylalanine (995 cm^{-1}) in Raman spectra and the amide I and amide II bands(1630, 1530 cm^{-1}) in IR spectra, an increase in DNA in both Raman (1083 cm^{-1}) and IR spectra(1080cm^{-1}). Regarding ciprofloxacin, an increase in nucleic acids (775, 1478, 1578 cm^{-1}) in Raman spectra and in protein bands (1230, 1520, 1630 cm^{-1}), in DNA (1080 cm^{-1}) in IR spectra were detected. Clear discrimination of antibiotic-treated samples compared to the control was recorded, showing that Raman and IR spectroscopies, coupled to principal component analysisfordata, could be used to detect molecular modifications in bacteria exposed to different classes of antibiotics. These findings contribute to the understanding of the mechanisms of action of antibiotics in bacteria.

Key words: Raman spectroscopy, Infra-red spectroscopy, *Escherichia coli*, antibiotics

1.Introduction

Antibiotics have been widely used for prevention and treatment of infectious diseases in humans and animals. However, their overuse has led to antimicrobial resistance among a wide range of infectious bacteria which become an increasing public health threat at global level (1) (2) (3) (4) (5) (6). According to recent statistics antibiotic-resistant infections currently involve about 700,000 deaths per year in the world (7). Among measures needed to tackle this complex and multi-faceted problem, rapid and accurate detection of antibiotic resistant bacteria is important to guide appropriate and efficient therapeutic treatments.

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In fact, conventional bacterial identification methods requiring selective cultures under a variety of conditions and morphological, biochemical evaluations of the cells are relatively laborious, costly and time consuming. In this context, it is crucial to develop alternative methods that offer real-time and reliable detection, identification of pathogenic bacteria, and discrimination of their susceptibility toward antibiotics. In this regard, Raman scattering and Infra-red spectroscopies (IR) are rapidly gaining ground in innovative research. Raman and IR spectroscopies provide significant benefits as non-invasive, non-destructive, reagent free and rapid diagnostic tools for single cells. The complete molecular pattern of the microorganisms is reflected in the Raman and IR spectra including different vibrational modes of compounds found in the bacterial cells (DNA/RNA, proteins, lipids, and carbohydrates…) (8) (9) (10). Consequently, Raman and IR spectroscopies have been used for several applications: identification of microorganisms (8) (11) (12) (13) (14) (15) (16) (17) (18), discriminating pathogenic and nonpathogenic bacteria (19). They have also shown a potential for the detection of antibiotic effects on the bacterial growth (20) (21) (22) (23), and the assessment of metabolic states and cell viability (24) (25) (26) (27).

Raman and IR are associated to different physics processes of interaction of radiation with matter but both provide vibrational information, showing different advantages. Raman spectroscopy is especially noted for investigations of biological samples, as it exhibits simple sample preparation and no influence of water medium. Additionally it is sometimes reported in literature that more spectral features are observed in Raman spectrum than in an IR spectrum over the same wavenumber range (28) (29) (30). However, using a combination of both techniques, Raman and IR, is powerful since both are complementary.The vast majority of molecules exhibited infrared bands in the mid-infrared region between 400 and 4000 cm^{-1}. A standard Raman spectrum also comprises the spectral range of 0 – 3500 cm^{-1}. The fingerprint region ranging from 400 to 1800 cm^{-1} is significant for the discrimination of microorganisms at the strain level (13) (24) (25) (31) (32) (33). In addition, the spectral range from 2700 to 3100 cm^{-1} is assigned to the C-H stretching modes originating from fatty acid chain of lipopolysaccharide, phospholipid, and the amino-side chains of proteins (31) (34) (35).

The present work aimed to use Raman (400 – 1800 cm^{-1}) and IR (1900 – 500 cm^{-1}) spectroscopies combined with principal component analysis to detect molecular changes induced by antibiotics in *Escherichia coli*.

2. Material and methods

2.1. Bacterial strain and growth conditions

Escherichia coli TOP10 (*E.coli* TOP10) strain (provided by CBAC research group -

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GEPEA - University of Nantes – La Roche/Yon, France) was grown overnight in nutrient agar at 37°C. Isolated colonies were transferred to 5 ml of sterile saline water in order to obtain a bacterial suspension with an optical density at 600 nm (OD_{600}) close to 0.1 (~ 10^8 CFU/ml). This suspension was then transferred into Mueller Hinton broth (1% v:v) at the final volume of 50 ml and then incubated at 37°C with orbital shaking at 200 rpm. Different growth phases (lag, exponential and stationary phase) of the bacterial strain were determined by measuring the OD_{600}. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of ampicillin (Amp), cefotaxime (Ctx), tetracycline (Tet), ciprofloxacin (Cip) against *E.coli* TOP10 were determined by macrodilution method (36).

2.2. Bacterial cultures with antibiotics

In order to detect molecular changes induced by antibiotics, the bacterial culture was prepared as described above. Ampicillin (Amp), cefotaxime (Ctx) and tetracycline (Tet) (Sigma Aldrich) were added to the bacterial culture at an OD_{600} value comprised between 1.5 and 2.0 (after 5h30 – 6h of incubation) (~ 10^7 - 10^8 CFU/ml) achieving a final concentration of 40 µg/ml, 1.6 µg/ml and 40 µg/ml respectively (~ 8MIC). In case of Cip, this antibiotic was added at the final concentration of 0.8 µg/ml (~ 8MIC) when the OD_{600} value of the bacterial culture ranged from 0.5 to 0.8 (~ 10^6 CFU/ml) after 4h

– 4h30 of incubation which gave more significant molecular changes in Raman and IR spectra than 5h30 – 6h. The cell inactivation after antibiotic treatments was calculated using the standard plate counts(CFU/ml) at the end of the exposure time for the control and treated samples (120 minutes after Amp, Ctx and Tet addition, and 90 minutes after Cip addition), expressed as percentage of bacterial count reduction (CFU/ml) and performed in triplicate.

2.3. Raman and IR absorption spectroscopy monitoring bacterial growth

Vibrational spectroscopy was used to monitor the metabolic changes occurring during bacterial growth. *E. coli* culture was prepared as described above. Raman and IR spectra were recorded at various growth times, mainly during the exponential (3, 6, 8 h of incubation) and stationary phases (24 h of incubation).

2.4. Sample preparation for Raman and IR

At the end of each antibiotic treatment, 10 ml of each bacterial culture were recovered by centrifugation at 8000 rpm for 5 min. The supernatant was discarded and the precipitate was suspended in sterile deionized water. In order to completely remove components of the Mueller Hinton broth, the bacterial cells were washed three times with sterile deionized water. Mueller Hinton medium is proven to be the best medium for both Raman spectroscopy and bacterial culture with antibiotics (37). The precipitate was then suspended in sterile deionized water for Raman and IR analysis (38).

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For Raman spectroscopy, 100 μl of this bacterial suspension (10 droplets/sample) were dropped onto a glass microscope slide, and then were air-dried for 5 – 10 min at room temperature (~ 20°C) with air humidity of 70 %. The nominal power of the laser was 5 mW which allows avoiding damage to the bacterial cells.

For IR spectroscopy, 3 ml of sterile water were added to the pellet. Then, the bacterial suspension was filtered through an aluminum oxide membrane filter (0.2 μm pore size, 25 mm diameter) (Anodisc, Whatman) using a Whatman vacuum glass membrane filter holder to collect bacterial cells. The anodisc membrane filter does not contribute to significant spectral features between the wavenumber of 4000 to 400 cm^{-1} and provides a smooth and flat surface onto which the bacterial film can form (30). The filter was dried at room temperature under a laminar air flow for 90 min before IR measurement. The control samples (antibiotic-free cultures) were prepared in similar conditions.

2.5. Spectral acquisition and processing

Micro- Raman spectra were measured in the range 400 – 1800 cm^{-1} with an excitation wavelength of laser at 638 nm using a confocal Raman XploRA ONE^{TM} spectrometer (JobinYvon, Horiba) with approximately 4 cm^{-1} resolution. The system was calibrated and monitored using a silicon reference (520.2 cm^{-1}) before the measurement. The accumulation time for each acquisition was 40 s and 3 accumulations were collected for a single measurement. Twenty spectra were recorded for each sample and the experiment was performed in triplicate.

IR absorption spectra were recorded in the range 4000 – 400 cm^{-1} using Bruker Vertex 70v spectrometer with 4 cm^{-1} resolution. The accumulation time for each acquisition was about 9 s and twenty accumulations were collected for a single measurement. Fifty spectra were obtained for each sample. In order to overcome the problem with water absorbance, the IR absorption spectra were measured in ATR mode (Attenuated Total Reflectance). From a general description the ATR accessory consists of a highly refractive element (in our case diamond crystal) which contacts the sample and allows the IR beam to reflect, leaving the evanescent wave to penetrate into the sample (39). In this work, it was used ATRaccessory purchased from Bruker company (Platinum ATR diamond F. vaccum – type A225/Q). This tool works in single reflection mode and samples are fixed using one-finger clamp mechanism.The bacterial cells were filtered by the anodisc membrane. This filter was placed between IR source and in contact with diamond crystal. The experiment was performed in triplicate.

2.6. Data analysis

Spectral processing included baseline removal and normalization using Labspec 6 for

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Raman spectra and Opus for IR spectra. For Raman spectra, the polynomial baseline correction and the vector normalization were applied. For IR spectra, the elastic baseline correction and the vector normalization were performed. Raman and IR spectral graphs were done by Origin 8.5.1 software. Statistic calculations using Principal Component Analysis method (PCA) were done with the help of The Unscrambler 10.2 software (CAMO). The spectral region of interest from 400 to 1800 cm^{-1} for Raman spectroscopy and 1900 to 500 cm^{-1} for IR spectroscopy were analyzed by PCA (13) (24) (25) (31) (32) (33) (40). The mean-centered data were analyzed by calculating the principal components (PCs), creating score plots for the first and second PCs. From the loadings of the principal components the wavenumbers having the highest variance can be identified.

3. Results and discussion

3.1. Bacterial growth monitored by Raman and IR

Fig. 1 showed representative Raman and IR spectra for different *E. coli* TOP10 growth phases after 3, 6, 8 and 24 h of incubation. Metabolic changes within bacteria as a function of growth time were reflected in changes in intensity of some spectral bands. According to the loading plot of the first PC accounting for 63% of the total variance for Raman and 66% for IR spectra (results not shown) the Raman spectral bands around 775 cm^{-1} (uracil and cytosine), 1571 cm^{-1} (guanine and adenine), 995

cm^{-1} (phenylalanine), 1087 cm^{-1} (DNA), 1445 cm^{-1} (lipid), 1660 cm^{-1} (protein) were identified to have the major contributions to the spectral variances during bacterial growth. Minor contributions resulted from the vibrational bands at 1230, 1533 and1636 cm^{-1} (amide III, amide II and amide I bands of protein), 1080 cm^{-1} (phospho-esters in DNA/RNA backbone) in IR spectra (41) (42) (43) (44) (45) (46).

Raman spectroscopy provides the chemical information of a variety of constituents within bacterial cells: DNA/RNA, carbohydrates, proteins, lipids. During the exponential phase (3, 6 and 8 hours after incubation) an increase in lipids, nucleic acids (DNA, RNA), proteins are necessary for the cell division and replication (25) (47). IR spectroscopy provides the overall chemical composition of the bacteria, with special focus on the protein components (especially due to intense peptide bond variations). It was found that during the exponential growth phase the protein content of the bacterial cells increased due to an enhanced synthesis of ribosomes for the translation and later due to an augmented synthesis of enzymes and other functional proteins. These findings were in agreement with other studies (24) (25) (26) (38) (40). Clear discrimination of the different growth time points (3, 6, 8 and 24 hours after incubation) by analyzing the score plot of the first and second PC was also observed.

3.2. Molecular changes induced by antibiotics

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The MIC and MBC values were equivalent for Amp, Ctx, Cip against *E.coli*TOP10 and corresponded to 5, 0.2 and 0.1 µg/ml, respectively. In case of Tet, the MIC and MBC were 5 µg/ml and 40 µg/ml, respectively. In order to produce significant inactivation of bacterial cells, the antibiotic concentration used in this study corresponded to 8MIC (40 µg/ml, 1.6 µg/ml, 40 µg/ml and 0.8 µg/ml for Amp, Ctx, Tet and Cip, respectively). Several antibiotic concentrations corresponding to 2MIC, 4MIC, 6MIC, 8MIC were tested. The concentrations of 2MIC, 4MIC and 6MIC did not provide significant molecular modifications in *E. coli* cells (data not shown). Finally, the concentration of 8MIC was chosen to perform the experiments because it was high enough to cause significant molecular changes in the bacterial cells. Compared to the control (100%), the inactivation of viable cells after the antibiotic addition were approximately 99.99 % for Amp, Ctx, Cip and 90% for Tet. It is likely that such stressful environment might induce modifications of the *E.coli* population that were detected in Raman and IR spectra.

Fig. 2 showed representative Raman and IR spectra for Amp, Ctx, Tet treatments after an incubation time of 120 minutes and the control (antibiotic-free culture). According to the loading plot of the first and second PCs, a decrease in proteins in both Raman (the amide III band – 1240 cm^{-1} and amide I band - 1660 cm^{-1}) and IR (the amide III band – 1230 cm^{-1},

amide II – 1530 cm^{-1} and amide I – 1630 cm^{-1}), an increase in carbohydrates (1150 cm^{-1}, 1020 cm^{-1}) in IR spectra were detected for Amp and Ctx treatments [24] [43]. Clear discrimination of antibiotic-treated samples compared to the control was recorded as well (Fig. 3). The spectral perturbation seems to be correlated with the mode of action of these drugs. Ampicillin and cefotaxime belong to β–lactam antibiotics that are inhibitors of cell wall synthesis. These drugs inhibit transpeptidation, the reaction that results in the cross-linking of two glycan-linked peptide chain. As a result, a newly synthesized bacterial cell wall is no longer cross-linked and cannot maintain its strength, leading to cell death [47] [48] [49] [50]. This can probably explain the decrease of the protein content observed for both Amp and Ctx treatments. Additionally, in order to repair damaged cell wall induced by antibiotics the bacteria may enhance the synthesis of precursors of peptidoglycan – N-acetyl glucosamine and N-acetyl muramic acid, generally amino-sugar. Consequently, the accumulation of these precursors inside the cells has led to the increase of the carbohydrate bands in IR spectra [20].

When compared to the spectrum of the control, some spectral changes were detected in the Raman and IR spectra of *E.coli* cells treated with Tet. The loadings of the first PC indicated the most prominent spectral differences including [40] [43]:

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- An increase of nucleic acid bands around 775 cm^{-1} (uracil, cytosine), 1478 cm^{-1} and 1578 cm^{-1} (guanine, adenine) in Raman spectra, and the amide III at 1230 cm^{-1} in IR spectra.

-An increase in DNA in both Raman (1083 cm^{-1}) and IR spectra (1080 cm^{-1}).

- A decrease of the amide I band in both Raman (1660 cm^{-1}) and IR (1630 cm^{-1}) spectra, the amide II band (1530 cm^{-1}) in IR spectra.

- A sharp decrease around 995 cm^{-1} (phenylalanine) in Raman spectra.

Tetracyclines are broad-spectrum agents, exhibiting activity against a wide range of Gram- positive and Gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae and protozoan parasites. Tetracycline reversibly inhibits bacterial protein synthesis by preventing the association of aminoacyl-tRNA with the ribosomal acceptor (A) site (51) (52) (53). The variations of Raman and IR spectra result from the mode of action of Tet against bacteria. When Tet attaches to the site A of ribosome, the protein synthesis is blocked which has led to a sharp increase of nucleic acids (775, 1478 and 1578 cm^{-1}) in Raman spectra and a decrease of the amide I and amide II bands (1630 and 1530 cm^{-1}) in IR spectra. Besides, an increase of the amide III at 1230 cm^{-1} in IR could result from enhanced synthesis of transport proteins such as efflux pump that are involved in the extrusion of antibiotics (54) (55).In addition, Tet could influence the synthesis of phenylalanine in

animal and microorganisms (52) (53) (56) (57) (58). Consequently, a dramatic decrease of phenylalanine band (995 cm^{-1}) in Raman was observed after the drug treatment. It was rather well demonstrated in PCA calculations shown on Fig. 3.

In case of Cip treatment, the largest spectral changes occurred, as shown in Fig. 4, in the wavenumber regions around 775 cm^{-1} (uracil, cytosine), 1478 cm^{-1}, 1578 cm^{-1} (guanine, adenine), 1087 cm^{-1} (DNA) in Raman spectra and 1230 cm^{-1}, 1520 cm^{-1}, 1630 cm^{-1} (amide III, amide II and amide I bands of proteins), 1080 cm^{-1} (DNA) in IR absorption spectra (41) (42) (43) (44) (45) (46) (59). Ciprofloxacin belongs to the second generation of quinolone analogues of nalidixic acid that show greater potency, lower toxicity and a broader antibacterial spectrum. In Gram-negative bacteria, Cip interferes with bacterial DNA gyrase and inhibits the DNA replication (47) (60) (61) (62) (63). As a result, a sharp increase in nucleic acid bands was recorded after Cip treatment. Furthermore, the spectral bands of proteins increased as well. This indicates that interactions of Cip with its cell targets and the subsequent reactions mainly involve changes at the DNA, but also proteins (40). The observed changes might not only be due to the pure action of the drug with it targets, but also caused by successive reactions (64) (65).

4. Conclusion

The findings clearly demonstrated that Raman and IR spectroscopies could be applied coupled with principal component analysis to detect *E. coli* metabolic changes at different growth phases and the molecular modifications induced by different classes of antibiotics. Some important spectral changes were determined under the influence of antibiotics: carbohydrate bands (1150, 1020 cm^{-1}), protein bands (1630, 1530, 1230 cm^{-1}) in IR spectra, nucleic acid bands (775, 1478, 1578 cm^{-1}), phenylalanine (995 cm^{-1}) in Raman spectra. It was then clearly shown that both Raman and Infra-red spectroscopies can contribute efficiently to the understanding of the mechanisms of action of antibiotics in bacteria and bring complementary information. This study could be the basis for future investigation for understanding the mechanisms involved in bacterial resistance to antibiotics using resistant strains.
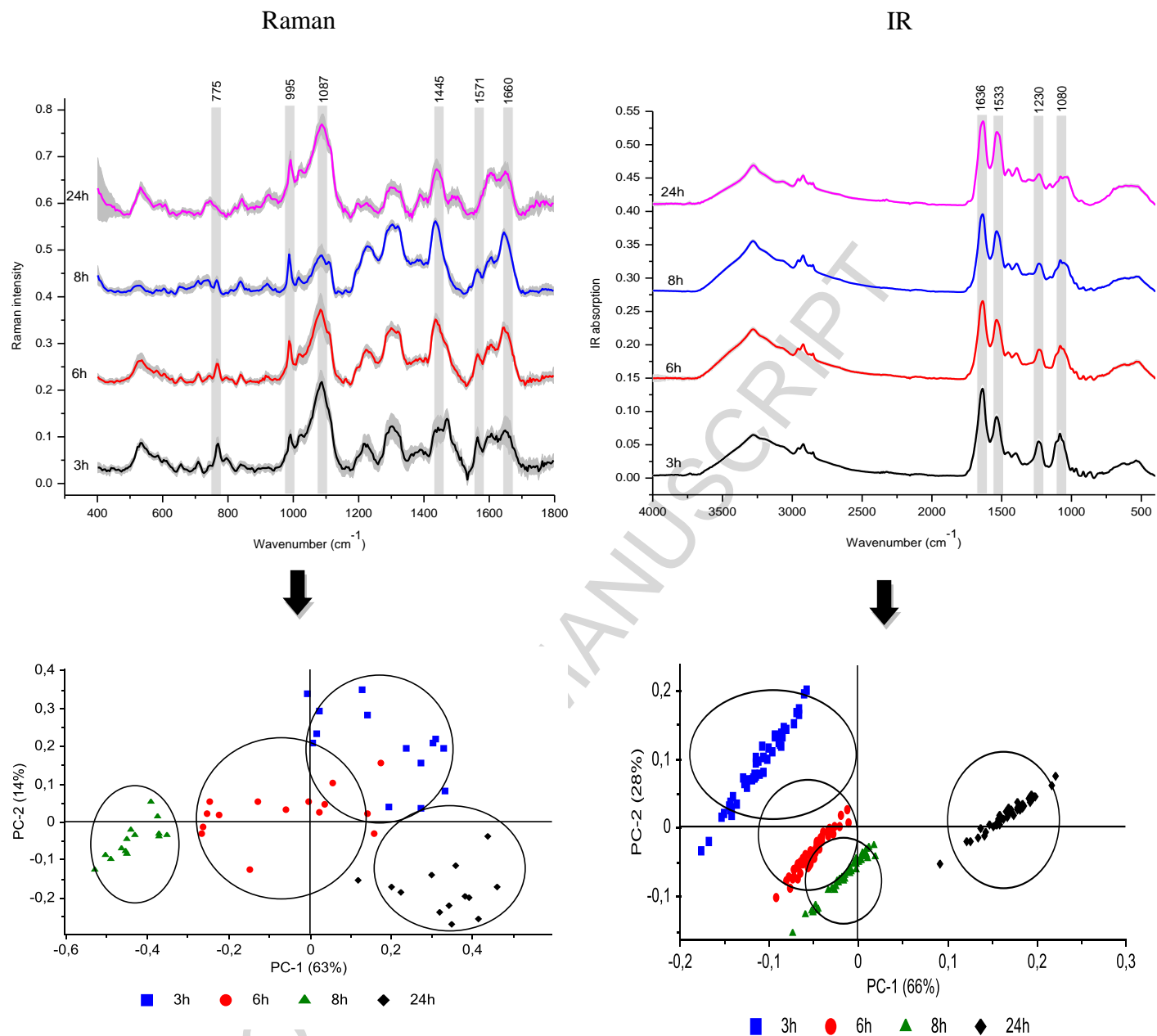
Acknowledgements

Fig. 1. Raman and IR spectra with double standard deviation as grey corona and the scores plot of the two first PC of *E. coli* TOP10 at different growth time points (3, 6, 8 and 24 hours after incubation)
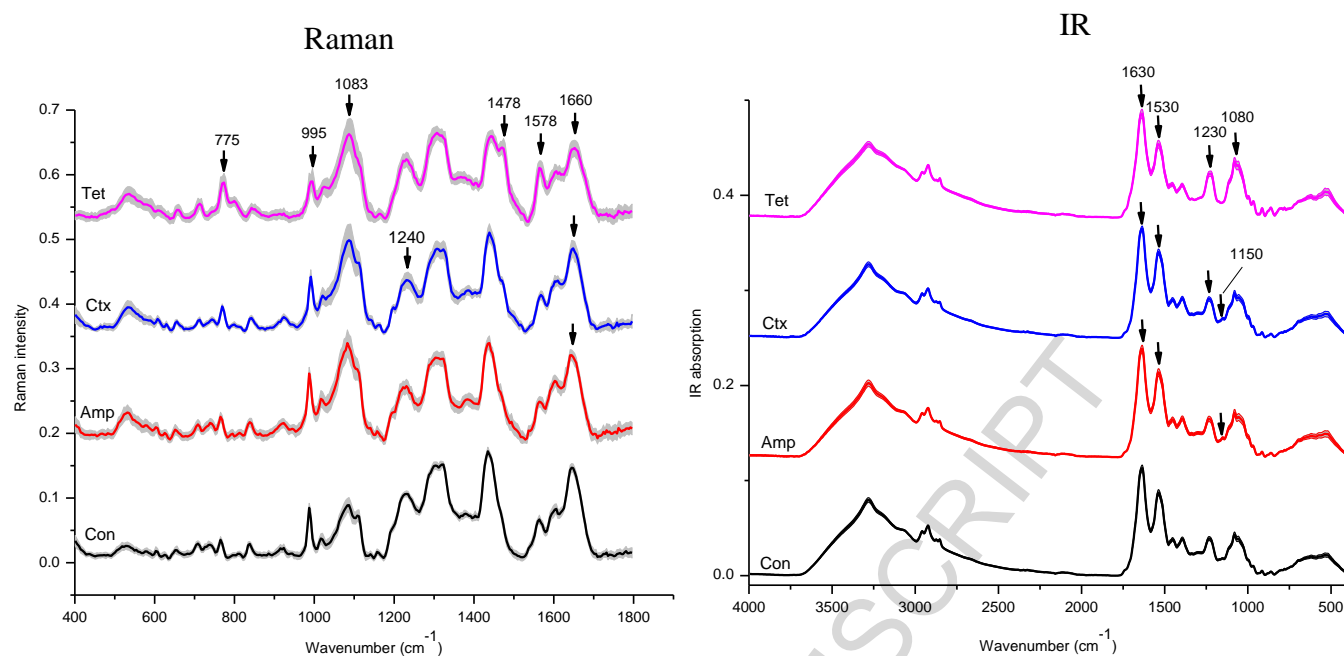
Fig. 2. Raman and IR spectra with double standard deviation as grey corona of *E. coli* TOP10 without (Control) and with Amp (40 µg/ml), Ctx (1.6 µg/ml) and Tet (40 µg/ml) recorded 120 minutes after the antibiotic addition
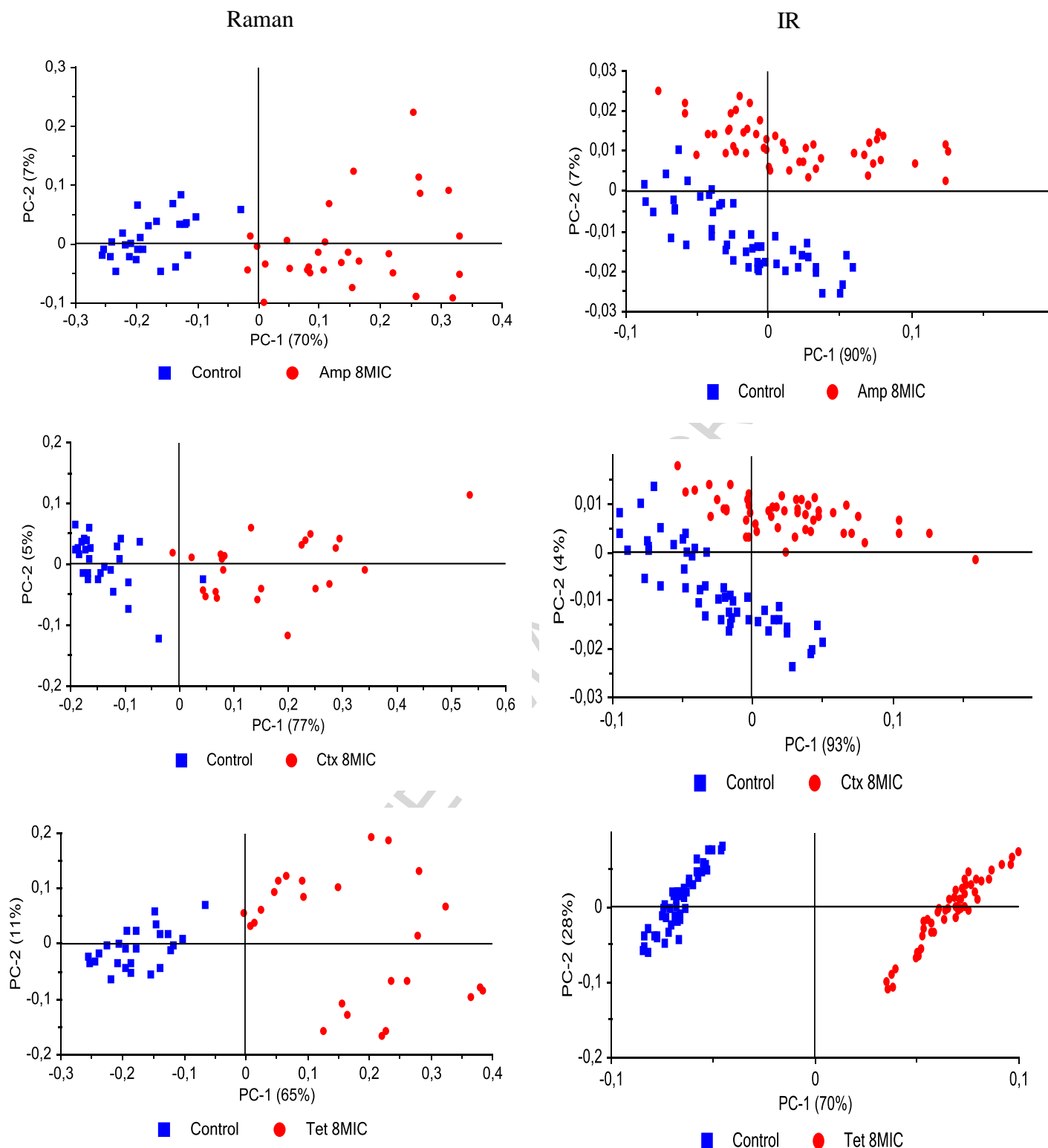
Fig.3. Scores plot of the first two principal components of Raman and IR spectra of *E. coli*TOP10 without (Control) and with Amp 8MIC (40 µg/ml), Ctx 8MIC (1.6 µg/ml) and Tet 8MIC (40 µg/ml) recorded 120 min after the antibiotic addition
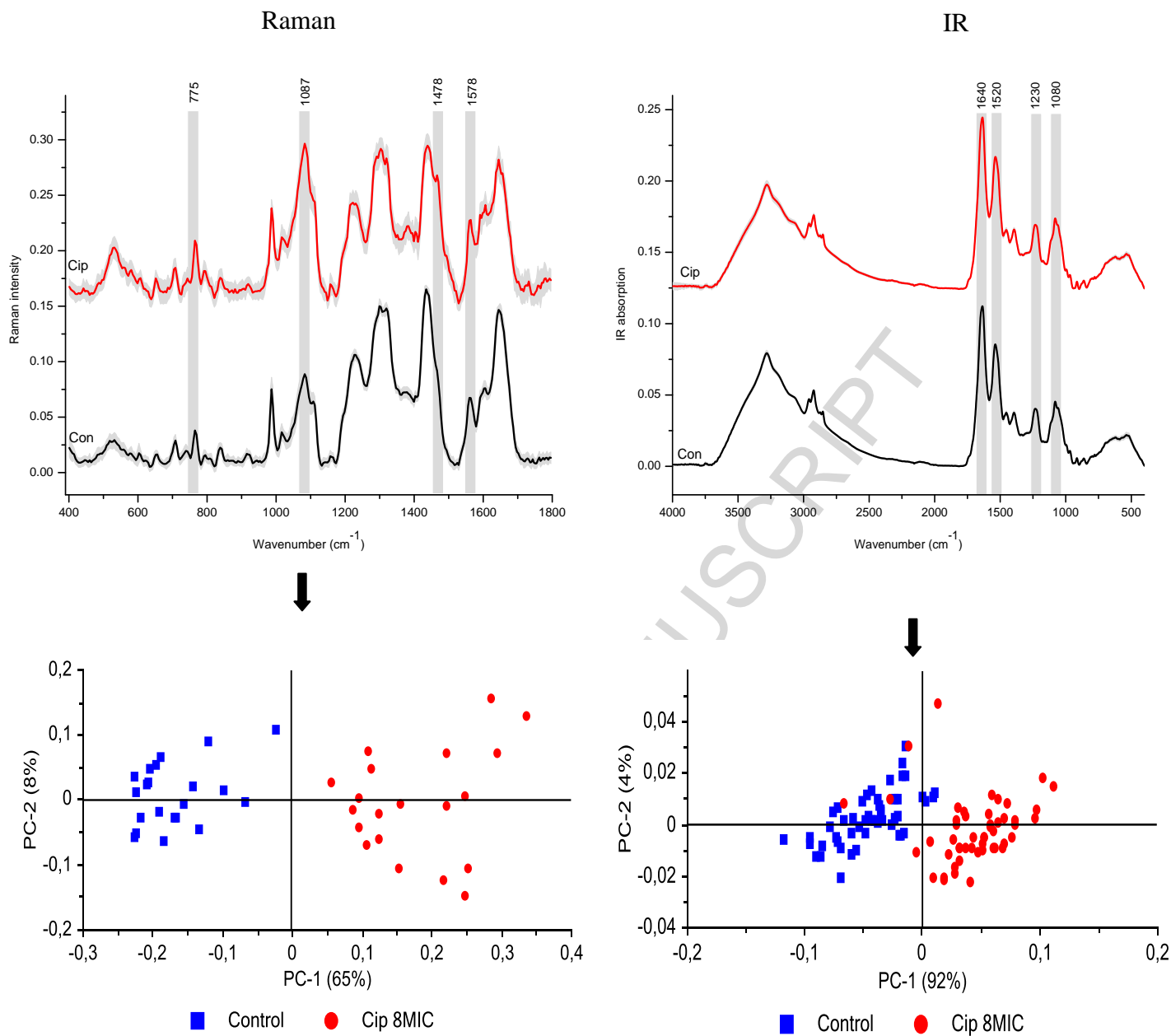
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Raman

IR

Fig. 4. Spectral graphswith double standard deviation as grey corona and scores plot of the first principal components for Raman and IR spectra of *E. coli* TOP10 without (Control) and with Cip (0.8 µg/ml)recorded 90 minutes after the antibiotic addition

References

[1] T. P. Van Boeckel, C. Brower, M. Gilbert, B. T. Grenfell, S. A. Levin, T. P. Robinson, A. Teillant and R. Laxminarayan, Global trends in antimicrobial use in food animals, PNAS 112 (2015) 5649–5654. DOI: 10.1073/pnas.1503141112.

[2] D. K. Pham, J. Chu, N. T. Do, F. Brose, G. Degand, P. Delahaut, E. De Pauw, C. Douny, K. V. Nguyen, T. D. Vu, M.-L. Scippo and H. F. L. Wertheim, Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. 2015, EcoHealth 12 (2015) 480-489. DOI: 10.1007/s10393-014-1006-z.

[3] R. R. Bonelli, B. M. Moreira, R. C. Picao, Antimicrobial resistance among Enterobacteriaceae in South America: History, current dissemination status and associated socioeconomic factors, Drug Resist. Updat. 17 (2014) 24-36.

[4] D. A. Tadesse, S. Zhao, E. Tong, S. Ayers, A. Singh, M. J. Bartholomew and P. F. McDermott, Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002, Emerging Infect. Dis. 18 (2012) 741-749. DOI: 10.3201/eid1805.111153.

[5] S.-H. Ryu, S.-G. Park, S.-M. Choi, Y.-O. Hwang, H.-J. Ham, S.-U. Kim, Y.-K. Lee, M.-S. Kim, G.-Y. Park, K.-S. Kim, Y.-Z. Chae, Antimicrobial resistance and resistance genes in *Escherichia coli* strains isolated from commercial fish and seafood, Int. J. Food Microbiol. 152 (2012) 14-18. DOI: 10.1016/j.ijfoodmicro.2011.10.003.

[6] S. Sarter, H. N. K. Nguyen, T. H. Le, J. Lazard, D. Montet, Antibiotic resistance in Gram-negative bacteria isolated from farmed catfish, Food Control 18 (2007) 1391–1396. DOI: 10.1016/j.foodcont.2006.10.003.

[7] C.-Y. Liu, Y.-Y. Han, P.-H. Shih, W.-N. Lian, H.-H. Wang, C.-H. Lin, P.-R. Hsueh, J.-K. Wang, Y.-L. Wang, Rapid bacterial antibiotic susceptibility test based on simple surface-enhanced Raman spectroscopic biomarkers, Scientific Reports 6 (2016) Article 23375. DOI: 10.1038/srep23375.

[8] S. Stöckel, J. Kirchhoff, U. Neugebauer, P. Rösch and J. Popp, The application of Raman spectroscopy for the detection and identification of microorganisms, J Raman Spectrosc 47 (2016) 89-109. DOI: 10.1002/jrs.4844.

[9] J. Schmitt, H.-C. Flemming, FTIR-spectroscopy in microbial and material analysis, Int. Biodeterior. Biodegradation 41 (1998) 1-11.

[10] A. Alvarez-Ordonez, D.J.M. Mouwen, M. Lopez, M. Prieto, Fourrier transform infrared spectroscopy as a tool to characterize molecular composition and stress response in foodborne pathogenic bacteria, J. Microbiol. Methods, 84 (2011) 369-378. DOI: 10.1016/j.mimet.2011.01.009.

[11] S. Pahlow, S. Meisel, D. Cialla-May, K. Weber, P. Rösch, J. Popp, Isolation and identification of bacteria by means of Raman spectroscopy, Adv. Drug Deliv. Rev. 89 (2015) 105-120.

[12] P. Whittaker, M. M. Mossoba, S. Al-Khaldi, F. S. Fry, V. C. Dunkel, B. D. Tall, M. P. Yurawecz, Identification of foodborne bacteria by infrared spectroscopy using cellular fatty acid methyl esters, J. Microbiol. Methods 55 (2003) 709-716. DOI: 10.1016/j.mimet.2003.07.005.

[13] D. Kusic, B. Kampe, P. Rosch, J. Popp, Identification of water pathogens by Raman microspectroscopy, Water Res. 48 (2014) 179-189.

[14] E. Kastanos, A. Kyriakides, K. Hadjigeorgiou and C. Pitris, A novel method for bacterial UTI diagnosis using Raman spectroscopy, Inter J Spectrosc 2012 (2011). Article ID 195317. DOI: 10.1155/2012/195317.

[15] S. Meisel, S. Stockel, P. Rosch, J. Popp, Identification of meat-associated pathogens via Raman microspectroscopy, Food Microbiol. 38 (2014) 36-43.

[16] M. A. Al-Holy, M. Lin, A. G. Cavinato, B. A. Rasco, The use of Fourier transform infrared spectroscopy to differentiate *Escherichia coli* O157:H7 from other bacteria inoculated into apple juice, Food Microbiol. 23 (2006) 162-168. DOI: 10.1016/j.fm.2005.01.017.

[17] P. Mobili, A. Londero, G. De Antoni, A. Gomez-Zavaglia, C. Araujo-Andrade, H. Avila-Donoso, R. Ivanov-Tzonchev and I. Moreno, C. Frausto-Reyes, Multivariate analysis of Raman spectra applied to microbiology: Discrimination of microorganisms at the species level, Revista Mexicana de Fisica 56 (2010) 378-385.

[18] J. P. Maity, S. Kar, C.-M. Lin, C.-Y. Chen, Y.-F. Chang, J.-S. Jean, T. R. Kulp, Identification and discrimination of bacteria using Fourier transform infrared spectroscopy, Spectrochim Acta Mol Biomol Spectrosc 116 (2013) 478-484.

[19] K. Hamasha, Q. I. Mohaidat, R. A. Putnam, R. C. Woodman, S. Palchaudhuri and S. J. Rehse, Sensitive and specific discrimination of pathogenic and nonpathogenic *Escherichia coli* using Raman spectroscopy—a comparison of two multivariate analysis techniques, Biomed Opt Express 4 (2013) 481-489.

[20] W. Zeroual, M. Manfait, C. Choisy, FT-IR spectroscopy study of perturbations induced by antibiotic on bacteria (*Escherichia coli*), Pathologie Biologie 43 (1995) 300-305.

[21] E. Kastanos, A. Kyriakides, K. Hadjigeorgiou and C. Pitris, Identification and antibiotic sensitivity of UTI pathogens using Raman spectroscopy, in P. Tenke (Ed) Urinary Tract Infections, InTech, 12 (2011) 207-226.

[22] X. Lu, B. A. Rasco, D.-H. Kang, J. M. F. Jabal, D. E. Aston and M. E. Konkel, Infrared and Raman spectroscopic studies of the antimicrobial effects of garlic concentrates and diallyl constituents on foodborne pathogens, Anal Chem. 83 (2011) 4137-4146. DOI: 10.1021/ac2001498.

[23] A. I. M. Athamneh, R. A. Alajlouni, R. S. Wallace, M. N. Seleem, R. S. Senger, Phenotypic profiling of antibiotic response signatures in *Escherichia coli* using Raman spectroscopy, Antimicrob. Agents Chemother. 58 (2014) 1302-1314.

[24] M. M. Hlaing, M. Dunn, P. R. Stoddart, S. L. McArthur, Raman spectroscopic identification of single bacterial cells at different stages of their lifecycle, Vib Spectrosc 86 (2016) 81-89.

[25] S.A. Strola, P.R. Marcoux, E. Schultz, R. Perenon, A.-C. Simon, I. Espagnon, C. P. Allier, J.-M. Dinten, Differentiating the growth phases of single bacteria using Raman spectroscopy, Biomedical Vibrational Spectroscopy VI: Advances in Research and Industry, Proc. of SPIE 8939 (2014) Article 893905. DOI: 10.1117/12.2041446.

[26] H. M. Al-Qadiri, N. I. Al-Alami, M. Lin, M. Al-Holy, A. G. Cavinato and B. A. Rasco, Studying of the bacterial growth phases using fourier transform infrared spectroscopy and multivariate analysis, J Rapid Methods Autom Microbiol 16 (2008) 73-89.

[27] H. Zhou, D. Yang, N. P. Ivleva, N. E. Mircescu, S. Schubert, R. Niessner, A. Wieser and C. Haisch, Label-free in situ discrimination of live and dead bacteria by surface-enhanced Raman scattering, Anal. Chem 87 (2015). DOI: 10.1021/acs.analchem.5b01271.

[28] T. Vankeirsbilck, A. Vercauteren, W. Baeyens, G. Van der Weken, F. Verpoort, G. Vergote, J. P. Remon, Applications of Raman spectroscopy in pharmaceutical analysis, Trends Analyt Chem 21 (2002) 869-877.

[29] B. Singh, R. Gautam, S. Kumar, B. N. Vinay Kumar, U. Nongthomba, D. Nandi, G. Mukherjee, V. Santosh, K. Somasundaram and S. Umapathy, Application of vibrational microspectroscopy to biology and medicine, Current Science, 102 (2012) 232-244.

[30] X. Lu, H. M. Al-Qadiri, M. Lin, B. A. Rasco, Application of mid-infrared and Raman spectroscopy to the study of bacteria, Food Bioprocess Technol 4 (2011) 919-935. DOI: 10.1007/s11947-011-0516-8.

[31] D. Naumann, S. Keller, D. Helm, Ch. Schultz and B. Schrader, FT-IR spectroscopy and FT-Raman spectroscopy are powerful analytical tools for the non-invasive characterization of intact microbial cells, J Mol Struct 347 (1995) 399-406.

[32] K. Kairyte, Z. Luksiene, M. Pucetaite and V. Sablinskas, Differentiation of bacterial strains by means of surface enhanced FT-Raman spectroscopy, Lithuanian Journal of Physics 52 (2012) 276-283.

[33] W. R. Premasiri, D. T. Moir, M. S. Klempner, N. Krieger, G. Jones II and L. D. Ziegler, Characterization of the surface enhanced Raman scattering (SERS) of bacteria, J Phys Chem 109 (2005) 312-320.

[34] A. A. Kamnev, P. A. Tarantilis, L. P. Antonyuk, L. A. Bespalova, M. G. Polissiou, M. Colina, P. H. E. Gardiner, V. V. Ignatov, Fourrier transform Raman spectroscopic characterization of cells of the plant-associated soil bacterium Azospirillum brasilense Sp7, J Mol Struct, 536-564 (2001) 199-207. DOI: 10.1016/S0022-2860(00)00877-2.

[35] K. Czamara, K. Majzner, M. Z. Pacia, K. Kochan, A. Kaczor and M. Baranska, Raman spectroscopy of lipids: a review, J Raman Spectrosc 46 (2014) 4-20. DOI: 10.1002/jrs.4607.

[36] J. M. Andrews, Determination of minimum inhibitory concentrations, J. Antimicrob. Chemother. 48 (2001) 5-16.

[37] F. S. S. Oliveira, H. E. Giana, L. Silveira Jr., Discrimination of selected species of pathogenic bacteria using near-infrared Raman spectroscopy and principal components analysis, J Biomed Opt 17 (2012). DOI: 10.1117/1.JBO.17.10.107004.

[38] M. F. Escoriza, J. M. Vanbriesen, S. Stewart and J. Maier, Studying bacterial metabolic states using Raman spectroscopy, Appl Spectrosc 60 (2006) 971-976.

[39] J. R. Goodwin, Vibrational microspectroscopy of bacterial colonies, Queensland University (2006) Ph.D. dissertation.

[40] U. Neugebauer, Characterization of bacteria, antibiotics of the fluoroquinolone type and their biological targets DNA and gyrase utilizing the unique potential of vibrational spectroscopy, Friedrich-Schiller-Universitat Jena (2007) Ph.D. dissertation.

[41] K. C. Schuster, I. Reese, E. Urlaub, J. R. Gapes and B. Lendl, Multidimensional information on the chemical composition of single bacterial cells by confocal Raman microspectroscopy, Anal. Chem. 72 (2000) 5529-5534.

[42] W. E. Huang, R. I. Griffiths, I. P. Thompson, M. J. Bailey and A. S. Whiteley, Raman microscopic analysis of single microbial cells, Anal. Chem. 76 (2004) 4452-4458. DOI: 10.1021/ac049753k.

[43] K. Maquelin, C. Kirschner, L.-P. Choo-Smith, N. van den Braak, H. Ph. Endtz, D. Naumann, G. J. Puppels, Identification of medically relevant microorganisms by vibrational spectroscopy, J. Microbiol. Methods 51 (2002) 255-271.

[44] M. Harz, P. Rosch, K.-D. Peschke, O. Ronneberger, H. Burkhardt and J. Popp, Micro-Raman spectroscopic identification of bacterial cells of the genus *Staphylococcus* and dependence on their cultivation conditions, Analyst 130 (2005) 1543-1550.

[45] M. Harz, P. Rosch, J. Popp, Vibrational spectroscopy - a powerful tool for the rapid identification of microbial cells at the single-cell level, Cytometry 75A (2009) 104-113.

[46] A. L. Jenkins, R. A. Larsen, T. B. Williams, Characterization of amino acids using Raman spectroscopy, Spectrochim Acta A 61 (2005) 1585-1594. DOI: 10.1016/j.saa.2004.11.055.

[47] M. T. Madigan, J. M. Martinko, K.S. Bender, D.H. Buckley and D. A. Stahl, Brock of biology microorganisms, thirteen ed., Bennjamin Cummings, 2012.

[48] B. W. Bycroft and R. E. Shute, The molecular basis for the mode of action of beta-lactam antibiotics and mechanisms of resistance, Pharm. Res. 2 (1985) 3-14.

[49] F. Malouin and L. E. Bryan, Modification of penicillin-binding proteins as mechanisms of beta-lactam resistance, Antimicrob. Agents Chemother. 30 (1986) 1-5.

[50] J. Ruiz, Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection, J. Antimicrob. Chemother. 51 (2003) 1109-1117. DOI: 10.1093/jac/dkg222.

[51] M. C. Roberts, Tetracycline therapy: Update, Clin. Infect. Dis. 36 (2003) 462-467.

[52] I. Chopra and M. Roberts, Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance, Microbiol. Mol. Biol. Rev. 65 (2001) 232-260. DOI: 10.1128/MMBR.65.2.232–260.2001.

[53] D. Schnappinger, W. Hillen, Tetracyclines: antibiotic action, uptake, and resistance mechanism, Arch Microbiol 165 (1996) 359-369.

[54] J. L. Martinez, M. B. Sanchez, L. Martınez-Solano, A. Hernandez, L. Garmendia, A. Fajardo, C. Alvarez-Ortega, Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. 2009, FEMS Microbiol Rev 33 (2009) 430-449. DOI: 10.1111/j.1574-6976.2008.00157.x.

[55] J. Anes, M. P. McCusker, S. Fanning and M. Martins, The ins and outs of RND efflux pumps in *Escherichia* coli, Front Microbiol 6 (2015). Article 587. DOI: 10.3389/fmicb.2015.00587.

[56] L. E. Day, Tetracycline inhibition of cell-free protein synthesis, J. Bacteriol. 92 (1966), 197-203.

[57] B. A. Freeman and R. Circo, Effect of tetracyclines on the intracellular amino acids of molds, J. Bacteriol. 86 (1963) 38-44.

[58] V. Ling and C. L. Morin, Inhibition of amino acid transport in rat intestinal rings by tetracycline, Biochim. Biophys. Acta 249 (1971) 252-259.

[59] M. F. Escoriza, J. M. VanBriesen, S. Stewart and J. Maier, Raman spectroscopic discrimination of cell response to chemical and physical inactivation, Appl Spectrosc 61 (2007) 812-823.

[60] J. M. Blondeau, Fluoroquinolones: Mechanism of action, classification, and development of resistance, Surv Ophthalmol 49 (2004) Suppl. 2, S73-S78. DOI: 10.1016/j.survophthal.2004.01.005.

[61] K. Drlica, Mechanism of fluoroquinolone action, Curr Opin Microbiol. 2 (1999) 504-508.

[62] R. J. Carman, M. A. Simon, H. Fernandez, M. A. Miller, M. J. Bartholomew, Ciprofloxacin at low levels disrupts colonization resistance of human fecal microflora growing in chemostats, Regul. Toxicol. Pharmacol. 40 (2004) 319-326. DOI: 10.1016/j.yrtph.2004.08.005.

[63] V. F. Samanidou, C. E. Demetriou, I. N. Papadoyannis, Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, and ciprofloxacin, in pharmaceuticals and blood serum by HPLC, Anal Bioanal Chem 375 (2003) 623-629. DOI: 10.1007/s00216-003-1749-9.

[64] M. Zeitlinger, R. Sauermann, M. Fille, J. Hausdorfer, I. Leitner and M. Muller, Plasma protein binding of fluoroquinolones affects

antimicrobial activity, J. Antimicrob. Chemother. 61 (2008) 561-567. DOI: 10.1093/jac/dkm524.

[65] S. Nissankararao, A. Reddy, Protein binding study of ciprofloxacin using semipermeable membrane, Int. J. Inv. Pharm. Sci., Vol. 1 (2013) 22-24.
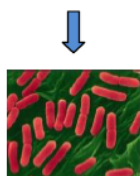
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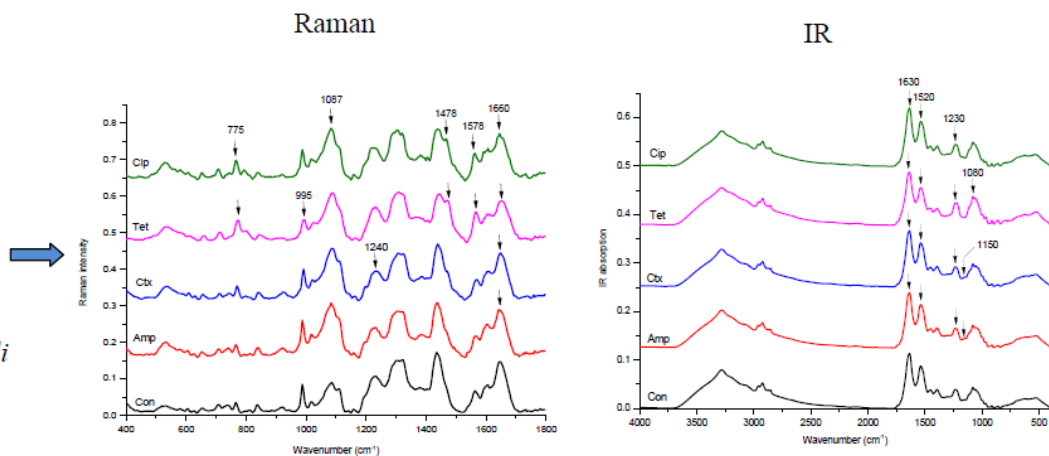
-Ampicillin, Cefotaxim

-Tetracycline

-Ciprofloxacin

Raman and IR spectra of *E. coli* TOP10 without (Con) and with Amp, Ctx, Tet recorded 120 minutes and Cip recorded 90 minutes after the antibiotic addition

Raman and IR spectra of *E. coli* TOP10 without (Con) and with Amp, Ctx, Tet recorded 120 minutes and Cip recorded 90 minutes after the antibiotic addition

Highlights

- Raman, IR were used to detect metabolic changes during growth phases of *E. coli*
- Metabolic changes in nucleic acids, lipids and proteins were observed
- Molecular changes induced by Amp, Ctx, Tet and Cip were detected
- Spectral discrimination by nucleic acids, proteins, phenylalanine in Raman spectra
- Spectral differentiation by carbohydrate, and protein bands in IR spectra