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Analysis of Changes in Envelope Cell of *Escherichia Coli Pathogenic* After Exposure to Stress Conditions Using FTIR

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Abstract: *Escherichia coli (E. coli)* pathogenic strains are causing foodborne illness, which represent a public health threat that must be confronted. Since bacterial cells react to different conditions of stress inducing physiological and structural changes, causing cells death. The aim of this study was to evaluate infrared spectral changes after exposure to different concentrations of detergent, metanol, polimixina B and proteinase K like factors of stress in the cell envelope of *E. coli*. For detergent, marked changes in profile of band PO_2- at 1251-1319 cm^{-1} were observed. Spectral variations corresponding to *E. coli* treated with methanol are observed at 1300 and 900 cm^{-1} and are associated to damage of the wall and cell membrane, when this cell was treatment with polimixina B, shows significant changes in the two sugar bands at 1070 to 1090 and 1030 to 1060 cm^{-1}. Finally, the effects of proteinase K on *E. coli* are observed mainly in amide I group and amide II groups, at 1800 and 1500 cm^{-1}, belonging to the proteins and peptides, which give us comprehensive information about proteins structure. Infrared results show that is possible to determine the effect of several stress concentrations associated to changes in the infrared absorption bands.

Keywords: *E.coli*, stress, infrared, cell, envelope, changes.

INTRODUCTION

Escherichia coli is an enteric species that distinguish to form part of the normal intestinal flora human, in fact some of these species supplies many health benefits to the host; for example, they prevent colonization

of the gut by harmful pathogens. However, there are small groups of *E. coli*, sometimes mentioned to as entero virulent *E. coli*, diarrheagenic *E. coli*, or more commonly, called pathogenic *E. coli*, that can cause severe diarrheal diseases in humans. Generally causing foodborne illness, which represent a public health threat that must be confronted, a way to address these problems is to have knowledge of what happens to bacteria when subjected to stressful conditions due to the presence of stress-injured bacterial cells in foods this understanding represents a challenge to those involved in food quality assurance. Given the fact that bacterial cells behave to the different environmental stress conditions by inducing structural and physiological changes, Fourier Transform Infrared (FT-IR) spectroscopy could be an alternative technique as reflects the biochemical composition of the cellular constituents that include water, fatty acids, proteins, polysaccharides, and nucleic acids[1, 2], also should be able to monitor the changes occurring in bacterial cells in response to several food-related stress conditions. The potential of this methodology to detect and differentiate sublethally substances -injured and dead pathogenic *E.coli* cells and to discriminate between diverse treatment intensities has been highlighted[2, 3.]

The aimed of this study was to assess ultra-structural modifications and infrared (IR) spectral changes at different concentrations of stress exposure and to discriminate between injured and noninjured *E.coli* cells after their exposure to Detergent, methanol, Polymyxin B (PMB) and proteinase K.

METHODS

E.coli strain used in this study was obtained from the Spanish Type Culture Collection. The lyophilized cultures were resuscitated in medium Tripticasein Soja Broth (TSB; Oxoid) and incubated for 24 h at 37°C. Pure cultures were maintained on this medium agar (Oxoid) plates at 4°C. Precultures were prepared by transferring an isolated colony from a plate into a test tube containing 10 ml of sterile TSB followed by incubation at 37°C for 24 h. Flasks containing 30 ml of sterile TSB were inoculated with the preculture to a final cellular concentration of 10^6 cells/ml, and these cultures were incubated at 37°C for 24 h (the time at which cells are in a late stationary phase of growth) and then were used to determine the bacterial resistance to different factors stresses and to perform the FT-IR spectroscopy measurements. Control cells, resuspended in PBS as described above, and cells subjected to different treatments: Detergent, metanol, Polimixin B and proteinase K, at different points in concentration were harvested by centrifugation at 10,000 g for 5 min at 4°C and suspended in 10ml of PBS, placed in a ZnSe crystal, and dried for 25 min. Infrared spectra were obtained with an FT-IR (Bruker Model Vertex-70) in Reflectance Total Attenuate mode (ATR)[3.] Measurements were recorded over the wavenumber range from 3500 to 400 cm^{-1}; the spectral resolution was 4 cm^{-1}. The final spectra of the samples were achieved averaging 20 scans. To obtain reproducible data, a strict experimental protocol was established in relation to medium preparation, incubation time and temperature, cell harvesting conditions, sample preparation and FT-IR measurement. Therefore, experimental conditions (see below) as well as the measuring time and resolution were standardized for each FT-IR measurement. The FT-IR experiments were conducted by triplicate using three different fresh cultures and with three separate measurements for each experimental set (nine different spectra for each strain exposure).

RESULTS

To determine the influence of several stress conditions on the FT-IR spectra in *patogenic E.coli*, stationary-phase cells grown in TSB were treated with (i) Detergent, (ii) methanol, (iii) Polymyxin and (iv), proteinase K, in a concentration range of (1-1000µ/ml). Strong absorptions were detected in five spectral regions that characterize the major cellular constituents, in Figure 1A *E.coli* cells treatment with detergent observed marked changes in profile of band PO_2- at 1251-1319 cm^{-1}. It is known that PO_{2-} profiles are sensitive to hydration-dehydration of the phosphate groups, and these can be taken as an indicator of the structural organization of lipids.
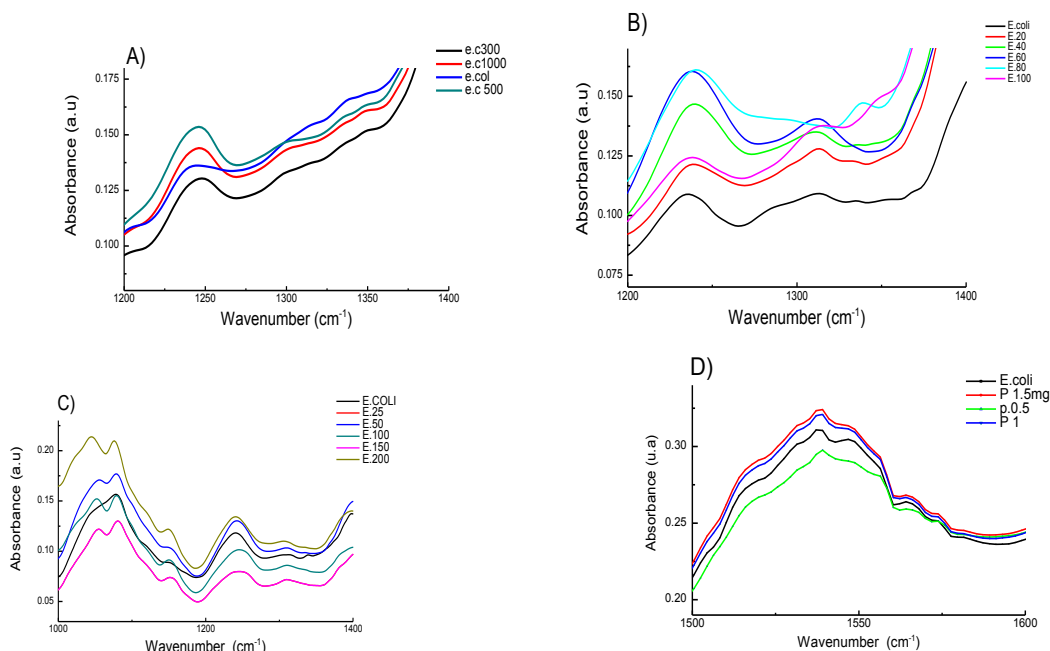
In Figure 1B, for Methanol-treated cells, showed important variations in the shape and intensity of several spectral bands. Thus, a reduction in the intensity of the peak found at approximately 1400 cm^{-1} is present, which seems to be composed of two separate new bands, at 1411 and 1385 cm^{-1}; new peaks emerged at 1350 cm^{-1} to concentration of 80 µ/ml; and to the same concentration the band found at 1384 cm^{-1} was less intense. The spectrum of *E.coli* nontreated (control) cells was visually similar to the spectra reported in previous studies for this microorganism, and other bacteria[3, 4.]

In Figure 1C spectral variations corresponding to *E. coli* treated with various concentrations of PMB present significant changes in the two sugar regions at 1070 to 1090 and at 1030 to 1060 cm^{-1}, with a decrease in the wavenumber for the first band and complete disappearance and/or shift to higher wavenumbers for the second band, indicating that PMB has a high affinity of binding to *E. coli*. Finally, in Figure 1D is possible observes the effect of different Proteinase K concentrations on *E. coli* at 1800 and 1500 cm^{-1}, affected by amide I group and amide II group belonging to the proteins and peptides[3-5.]

These results indicates that some or all of the cell compounds, like polysaccharides of the cell wall, fatty acids of the cell membrane, proteins, nucleic acids, or other compounds of the cell body, show significant alterations during these inactivation treatments by absorb in these spectral regions. On the other hand, for proteinase k stress, the IR spectrum did not present significantly differences between control spectrum, which does not indicate the absence of a defensive bacterial response to this stressful agent (Figure 4D). Nevertheless, each treatment condition caused specific effects on the different spectral ranges. *E.coli* exposure to environments gave rise to minor effects on the phospholipids spectral region, which indicates the occurrence of small or undetectable alterations on membrane fatty acids.

Methanol and detergent stress were the stress conditions that modified the 1200-1400 cm^{-1} range to a higher concentrations, methanol to high concentrations treatment mainly affected the band at 1400 cm^{-1} due to the superposition of C-O-H in plane bending (1415 cm^{-1}) and $C(CH_3)_2$ stretching (1402 cm^{-1}). Carbohydrates, the DNA RNA backbone, and proteins all contribute to this band[6, 7.]

All the stress conditions have an effect on the FT-IR spectroscopic profiles, indicating a higher degree of membrane/wall damage or chemical modification after bacterial exposition to them. Nevertheless, it is important to keep in mind that the spectral region that showed the main changes, is in the range from 1200 to 1000 cm^{-1}, which is dominated by the ring vibrations of the functional groups C-O-C and C-O from the carbohydrates of the cell wall; the behavior showed in this region is quite similar when an acid treatment occurs[8, 9.]

Figure 1: Effect of different stress concentrations of *E. coli.* A) Detergent, B) methanol, C) Polymyxin B and D) proteinase K.

CONCLUSIONS

In conclusion, results obtained not only indicate the potential of FT-IR spectroscopy to discriminate between intact and injured bacterial cells and between different concentrations treatment, but also show the capacity of this technique to study the molecular aspects of the bacterial stress response. FT-IR spectroscopy is a physicochemical method that can determine the global chemical features of cells, and therefore it represents an adequate technique to study the molecular changes after stress exposure, since significant changes in the chemical composition of cells previously have been described in response to a wide variety of stress conditions, including heat, acid, alkaline, and oxidative stress. Results obtained also could help us improve our knowledge of pathogenic *E.coli* cell damage and strategies of response to these adverse conditions.

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REFERENCES

1. H.M. Al-Qadiri, M. Lin, A. G. Cavinato, and B. A. Rasco, Fourier transform infrared spectroscopy, detection and identification of *Escherichia coli* O157:H7 and *Alicyclobacillus* strains in apple juice. *Int. J. Food Microbiol, 2006,* 111: 73–80.

2. H.M. Al-Qadiri, M. Lin, M. A. Al-Holy, A. G. Cavinato, and B. A. Rasco. Detection of sublethal termal injury in *Salmonella enterica* serotype Typhimurium and *Listeria monocytogenes* using Fourier transform infrared (FT-IR) spectroscopy (4000 to 600 cm_1). J. *Food Sci*., 2008, 73:54–61.

3. A.T. Oust, K. Moretro, G.D. Naterstad, I. Sockalingum, I. Adt, M. Manfait, and A. Kohler, Fourier transform infrared and Raman spectroscopy for characterization of *Listeria monocytogenes* strains. Appl. Environ. Microbiol, 2006, 72:228–232.

4. A.Aˊlvarez-Ordoˊnˉez, A. Fernaˊndez, A. Bernardo, and M. Loˊpez, Comparison of acids on the induction of an acid tolerance response in *Salmonella* Typhimurium, consequences for food safety. *Meat Sci*., 2009, 81:65–70.

5. A. Alvarez-Ordoˊnˉez, J. Halisch, and M. Prieto, Changes in Fourier transform infrared spectra of *Salmonella enterica* serovars Typhimurium and Enteritidis after adaptation to stressful growth conditions. Int. *J. Food Microbiol*, 2010, 142:97–105.

6. M.C. Curk, F. Peladan, and J. C. Hubert, Fourier transform infrared (FT-IR) spectroscopy for identifying *Lactobacillus* species. FEMS Microbiol. Lett, 1994, 123:241–248.

7. J.Y.D' Aoust,*Salmonella*, p. 1233–1299. *In* B. M. Lund, T. C. Baird- Parker, and G. W. Gould (ed.), The microbiological safety and quality of foods. Aspen, Gaithersburg, MD, 2000.

8. Ede, S. M., L. M. Hafner, and P. M. Fredericks, Structural changes in the cells of some bacteria during population growth: a Fourier transform infrared-attenuated total reflectance study. *Appl. Spectrosc*.2004, 58:317–322.

9. D.J.M. Mouwen, M. J. B. M. Weijtens, R. Capita, C. Alonso-Calleja, and M. Prieto, Discrimination of enterobacterial repetitive intergenic consensus PCR types of *Campylobacter coli* and *Campylobacter jejuni* by Fourier transform infrared spectroscopy. Appl. *Environ. Microbiol*. , 2005, 71:4318–4324.

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