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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* 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.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.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). 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.

**Keywords:** *Escherichia coli,* physiological changes, Tripticasein Soja Broth.

## 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].

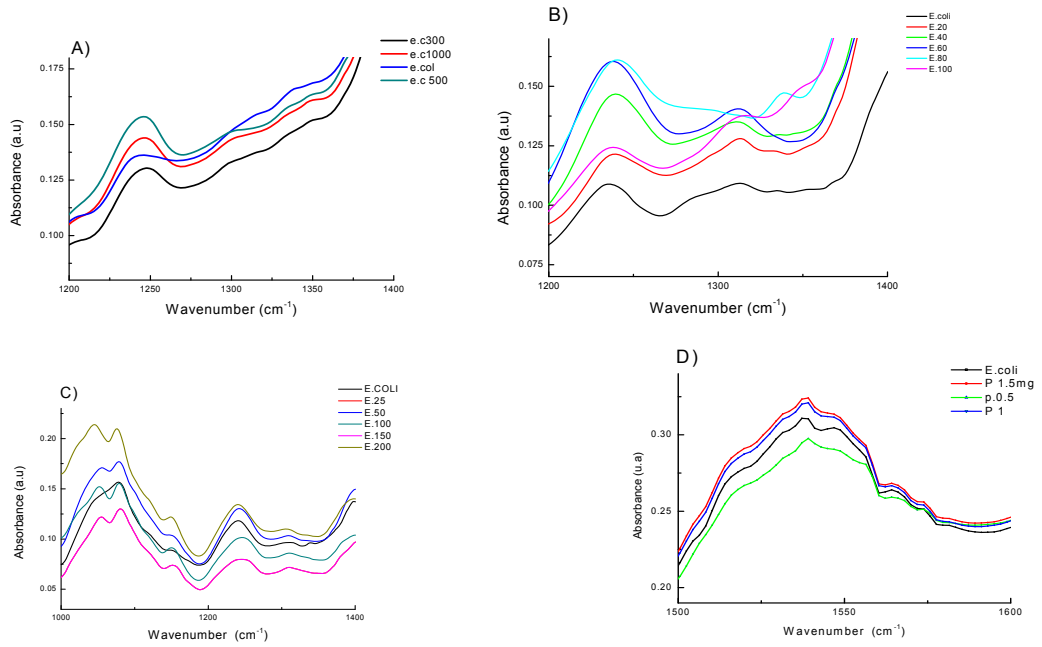


**Figure 1:** Effect of different stress concentrations of *E. coli*. A) Detergent, B) methanol, C) Polymyxin B and D) proteinase K.

## 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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