FOURIER TRANSFORM INFRARED SPECTROSCOPY APPLIED TO DETERMINATE THE CELLULAR COMPOSITION OF *Escherichia coli* UNDER DIFFERENT STRESS FACTORS

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Introduction. *Escherichia coli* is a pathogen that have caught the attention of researchers due to their economic impact when illnesses occur. To reduce the presence of these pathogens, different approaches have been used therefore, the investigation of the effectiveness of potential antimicrobials or stress factors is necessary. Fourier transform infrared (FT-IR) spectroscopy is known to offer the potential for studying the total molecular composition of cells[1] since all the functional groups of organic (bio)molecules specifically absorb infrared (IR) light. Thus, valuable information on the chemical composition of the cells can be obtained[2], FT-IR spectroscopy has been often used for taxonomy studies[3]. However, there are several reports on the application of this technique to detect changes at the cellular level after exposure of cells to different stress factors[4]. The aim of the present study was to use FT-IR spectroscopy in order to elucidate the changes caused by different stress factors on *Escherichia coli* at the cellular level.

Methods. Cells of *Escherichia coli* were centrifuged, washed twice with PBS buffer (pH 7.2), and resuspended in the same buffer. An aliquot of 500 µL (2×10^9 CFU/mL) of the suspension was incubated with 500µL of: detergent concentrated phosphate free, lysosome, protease K, methanol and control at different dilutions (10^{-1}-10^{-4}). After 24 h of incubation (37°C), cells were washed twice with PBS buffer (pH 7.2) for each dilution, CFU/mL were determine. The sample was measurement in a FT-IR spectrometer in ATR mode, according to Papadimitriou y cols. (2008)[5]. Spectra were recorded at 4 cm^{-1} spectral resolution and each spectrum was achieved by co-adding 120 scans.

Results. In figure 1 shows the different absorbance values corresponding to the several dilutions of culture of *E. coli,* the spectra shows two bands at 1540 cm^{-1} and other around 1641 cm^{-1} within the protein amide I band for 10^{-4} dilution, in contrast with the band at 946 cm^{-1} for the other dilutions the last one is attributed to C–H bond the lower dilutions present absorption between 1600 and 1300 cm^{-1} except in the amide I region, implying an increase of helical content in the proteins, in contrast figure 2 shows the effect of detergent concentrated phosphate on culture of *E.coli*. In both figures is possible observe that amide I and amide II bands of *E.coli,* from approximately 1700 to 1500 cm^{-1}, present a maximum near to 1620 cm^{-1} this band is attributed to C=O bond in the stretching vibration weakly mode, coupled with C–N stretching and N–H bending vibrations in the backbone of the amide group. However, the fine structure within the amide I band cannot be obtained directly in the original spectrum. The spectral behavior in the amide I region of the culture *E.coli* after detergent treatment shows changes in the band 1620 cm^{-1} associated to diminution of bacterial culture of *E. Coli*. In addition, the kinetic growth of the pathogen was determine also by FTIR in the same concentration of detergent, lysosome, protease K and methanol. The bactericidal effect of the different stress factors was validated and resulted in reduction of the CFU/ml of *Escherichia coli*.

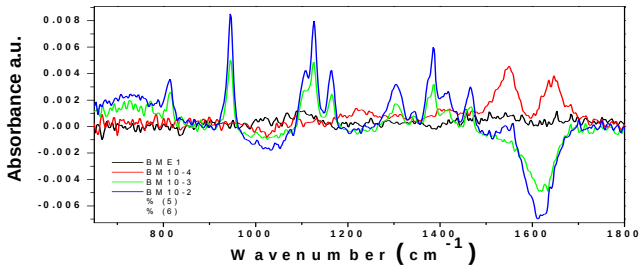

Fig. 1. Infrared spectra of Culture of *E.Coli* to different dilutions.

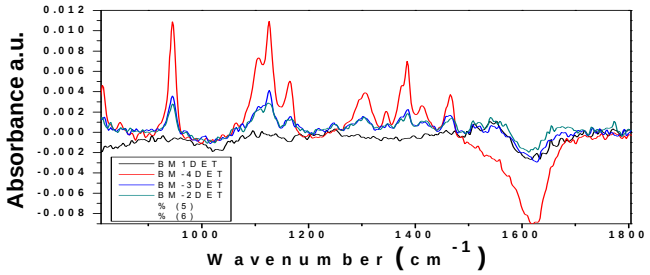

Fig. 2. Infrared spectra of culture of *E.Coli* treated with detergent phosphate free to different dilutions.

Conclusions. We show that with FTIR is possible observe the behavior associate to the cellular composition when *E. Coli* is under different stress conditions.

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References.

1. Helm, D., Naumann, D., 1995. *FEMS Microbiology Letters* 126, 75–79.

2 Oust, A., Moen, B., Martens, H., Rudi, K., Naes, T., Kirschner, C., Kohler, A., 2006. *Journal of Microbiological Methods* 65, 573–584.

3. Mariey, L., Signolle, J.P., Amiel, C., Travert, J., 2001. *Vibrational Spectroscopy* 26, 151–159.

4.- Kamnev, A.A., Sadovnikova, Yu.N., Antonyuk, L.P., 2008. *Microbiology (Moscow)* 77, 240–242.

5.- Papadimitriou, K., Boutou, E., Zoumpopoulou, G., Tarantilis, P.A., Polissiou, M., Vorgias, C.E., Tsakalidou, E., 2008. *Applied and Environmental Microbiology* 74, 6068–6076.