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# Use of FT-IR Technique as a Method for Differentiation of Bacterial and Fungal Strains

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**Abstract**.For this study we chose a series of commonly encountered bacterial and fungal microorganisms: *Escherichia coli, Pseudomonas aeruginosa, Pseudomonas putida F1, Klebsiella pneumoniae, Staphylococcus aureus, Micrococcus luteus* and *Candida albicans.* For their analysis using infrared spectrophotometry. Interpretation of data from the Spectrophotometer reading was done with the program Origin version 7. The results showed detectable differences between the spectra taken on bacteria and fungi. Could differentiate the chromatogram peaks characteristics for bacteria and fungi. The graphics made it was found that the combination of polysaccharide region (1200-900 cm-1) with "fingerprint region" (900-700 cm-1) and mixed region (1500-1200 cm-1) spectra and their derivatives primarily were most useful to characterize the FT-IR spectra, and to differentiate microorganisms. From the research done on the microorganisms: *Staphylococcus aureus, Micrococcus luteus, Pseudomonas putida F1, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans* can concluded that they can differentiate using FT-IR technique. FT - IR Identification techniques is a fast and accurate of microorganisms identification techniques (organisms composition differs, molecular composition in generals is different and FT-IR spectra of them will be different) with shortening of identification time. Method FT - IR can be used for rapid identification of microorganisms. Being only need a small amount of culture for 24 hours and 5 minutes to read Spectrophotometer IR light.

**Keywords:**FT-IR, bacteria, identification, strain, chromatograms, spectroscopy

## INTRODUCTION

In recent years we witnessed the emergence of new techniques, sensitive, rapid and more accurate for microbiological analysis. These new range of techniques left from various spectroscopic techniques such as molecular spectroscopy (including FT-IR and mass spectroscopy) to various separation techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC). (Nelson, 1985, 1991, Fox et al., 1990; Mantsch and Chapman, 1996).

FT-IR spectroscopy is a nondestructive technique and allows rapid and simultaneous characterization of complex materials such as bacteria (Helm et al, 1991, Naumann et al, 1991). For example, FT-IR was used as a type safe and easy method to identify and classify bacteria at the genus, including *Listeria* (Holt et al, 1995), *Staphylococcus., Clostridium, Streptococcus, Legionella, Escherichia coli* (Choo-Smith et al, 2001, MA Miguel et al, 2003, Naumann et al,. 1994), but also at the species, including *Pseudomonas, Bacillus* (MA Miguel et al, 2003;. Udelhoven et al, 2000), enterococi (Kirschner et al, 2001) and yeast, including *Candida* (Choo-Smith et al, 2001).

These studies show that it is possible to create differences between different organisms depending on genus, species, even strain level and studies have shown the ability to differentiate microorganisms from various groups (Helm et al., 1991).

## MATERIALS AND METHODS

For this study we have chosen a number of commonly encountered bacterial and fungal microorganisms: *Escherichia coli, Pseudomonas aeruginosa, Pseudomonas putida F1, Klebsiella pneumoniae, Staphylococcus aureus, Micrococcus luteus* and *Candida albicans*. Bacteria and fungi used were taken from the collection held by the Department of Microbiology University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. For their analysis using infrared spectrophotometry (Spectrophotometer IR Prestige - 21 Shimadzu).

For the FT - IR analysis, microorganisms found on liquid medium were passed into fresh culture medium. Bacteria were kept for 24 hours at $37^0$ C to 180 rpm. Reading tests were made on the photometer module ATR - IR. On ATR module an amount of 100µl of substance is stretched to make a thin and homogeneous film. For each sample introduced, the unit has made a number of 64 readings for an accurate result and noise reduction. After each reading ATR module was cleaned and washed with acetone to remove the broth and the studied microorganism. Data were retrieved and further processed with Origin Lab software version 7.

## RESULTS AND DISCUSSIONS

We wanted to identify microorganisms by using infrared light (IR) and thus obtain chromatograms for each organism individually and choosing the comparison group as similar phenotype organisms. Interpretation of data from the Spectrophotometer reading was done with the program Origin version 7. When interpreting these results were obtained:

**Micrococcus luteus - Staphylococcus aureus**

Comparing the chromatograms from *Micrococcus luteus* and *Staphylococcus aureus* note absorbance differences between 3750 - 2500 $cm^{-1}$ where there are groups NH, OH, $CH_2$ and $CH_3$. In figure 2 the interval between 1750-500 $cm^{-1}$ there are some similarities between the two organisms studied difference occurs around 969 cm-1 which is C-N +-C, and absorbance value differs from the two organisms, but remains somewhat the same wavelength throughout.

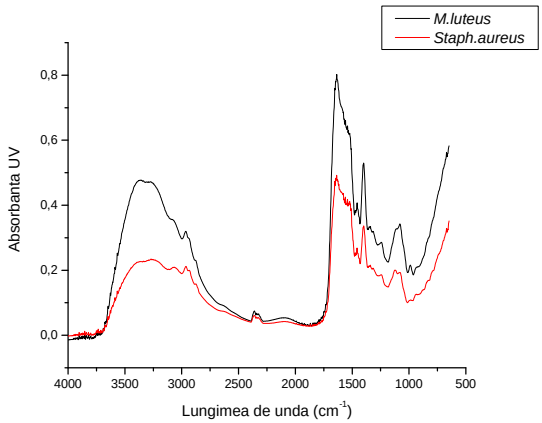


Fig.1. Chromatograms FT-IR representing *Micrococcus luteus* - *Staphylococcus aureus* measurement range 4000 – 500 $cm^{-1}$

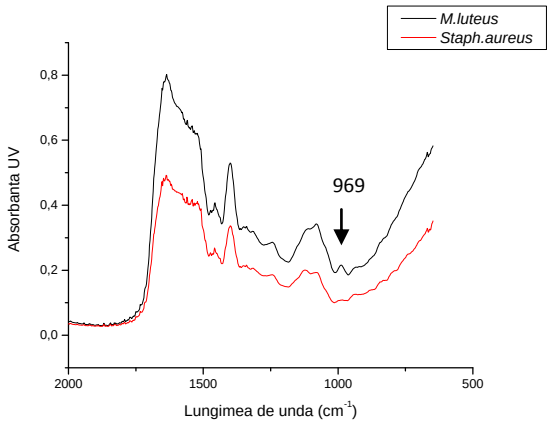


Fig.2. Chromatograms FT-IR representing *Micrococcus luteus* - *Staphylococcus aureus* measurement range 2000 – 500 $cm^{-1}$

***Pseudomonas putida F1- Pseudomonas aeruginosa***

Belong to the same genre but are two totally different species. *Pseudomonas aeruginosa* is found in soil, water and bacterial flora of the skin and can cause serious illness in humans and animals. *P. aeruginosa* is a bacterium that lives on the natural and artificial media, has a wide range of substrates that are used, to animals is known that infect the damaged tissue and attack people with low immune system. While *Pseudomonas putida F1* likes media rich in aromatic hydrocarbons, including benzene, toluene, ethylbenzene, it is a bacterium commonly used in bioremediation.

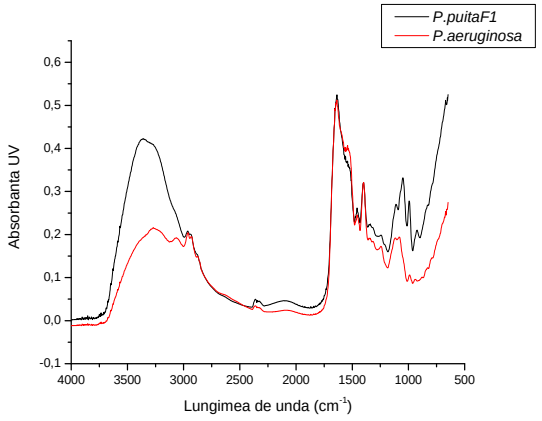


Fig.3. Chromatograms FT-IR representing *Pseudomonas putida F1- Pseudomonas aeruginosa* measurement range 4000 – 500 $cm^{-1}$

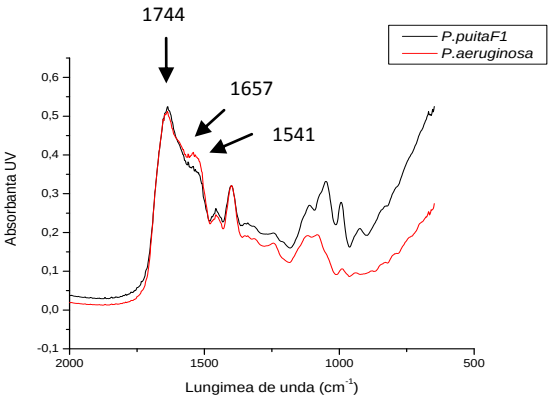


Fig.4 Chromatograms FT-IR representing *Pseudomonas putida F1- Pseudomonas aeruginosa* measurement range 2000 – 500 $cm^{-1}$

In the study of graphs from the review of FT - IR between *Pseudomonas putida F1*, *Pseudomonas aeruginosa* is observed differences in absorbance at different wavelengths.

Significant differences shown in figure 4 between the two organisms in the range of occurrence of the groups NH, OH, CH2 and CH3 the difference being the amplitude. Small differences in absorbance are esters (1739 - 1744 $cm^{-1}$), amide (1657 and 1541 $cm^{-1}$), the

largest differences between 1455-750 $cm^{-1}$ there are found links (CH2 1452 $cm^{-1}$ , COO-1391 $cm^{-1}$, $PO^{-2}$ 1236 and 1080 $cm^{-1}$, CO-OC 1152 $cm^{-1}$, C-$N^{+}$-C 969 $cm^{-1}$).

**Escherichia coli – Klebsiella pneumonia**

There are genres like part of the family *Enterobacteriaceae.* A small difference between the two organisms is observed at 1541 cm-1 which is grouping amides

The chromatogram represented in fig. 5 and 6 where is the *Escherichia coli* and *Klebsiella pneumoniae* is observed that the two organisms have similar absorbance measure all studied range 4500-500 $cm^{-1}$.

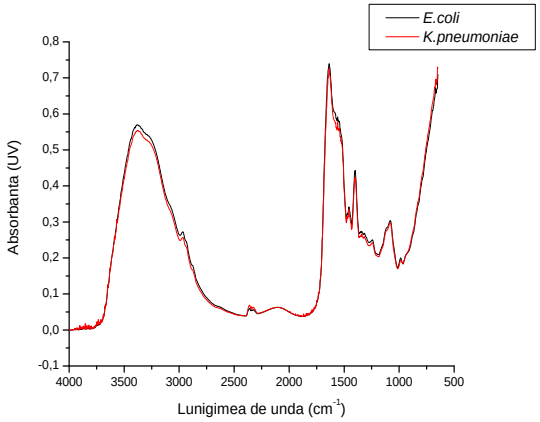


Fig.5. Chromatograms FT-IR representing *Escherichia coli – Klebsiella pneumoniae* measurement range 4000 – 500 $cm^{-1}$

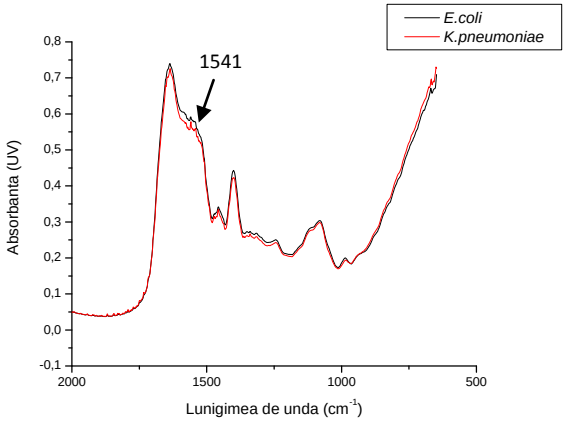


Fig.6. Chromatograms FT-IR representing *Escherichia coli – Klebsiella pneumoniae* measurement range 2000 – 500 $cm^{-1}$

For an overview of all 6 bacterial spectra have been superimposed To this was added the resulting spectrum analysis represented by Candida albicans fungi, but also gave witness to the culture medium.

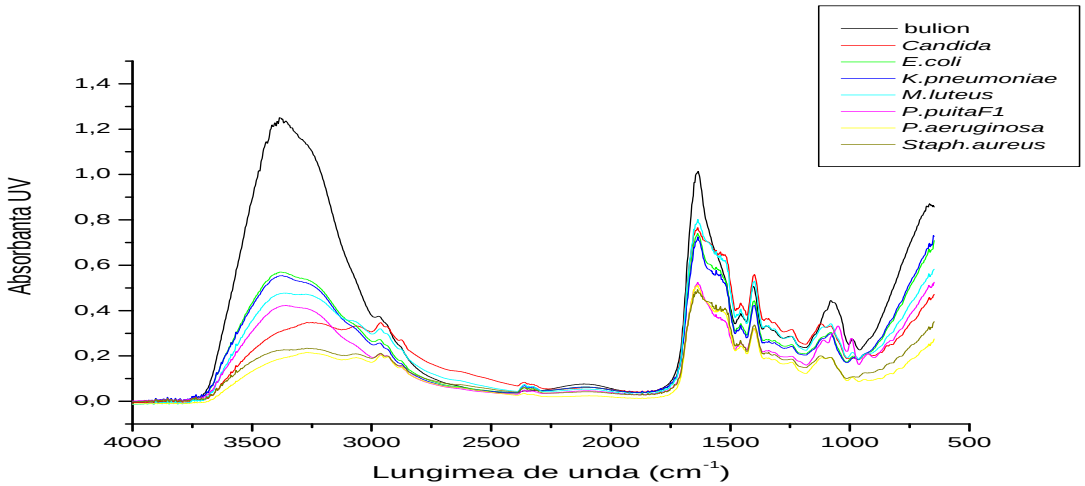


Fig.7. FT-IR chromatograms representing an overlap of microorganisms (*Staphylococcus aureus, Micrococcus luteus, Pseudomonas putida F1, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans*) on measurement range 4000 – 500 $cm^{-1}$

The results showed detectable differences between the spectra taken on bacteria and fungi. Could differentiate the chromatogram peaks characteristics to bacteria and fungi. It seems that this technique could be used to discriminate rapidly between bacterial infections and fungal contamination.

In the graphics made it was found that the combination of polysaccharide region (1200-900 cm-1) fingerprint region (900-700 cm-1) and mixed region (1500-1200 cm-1) spectra and their derivatives were most useful to characterize the FT-IR spectra of the selected microorganisms

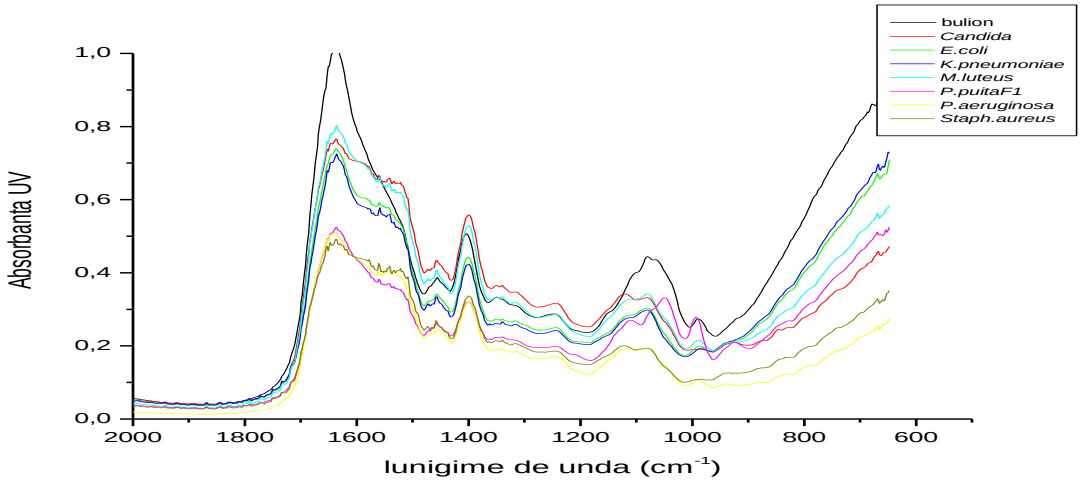


Fig.8. FT-IR chromatograms representing an overlap of microorganisms (*Staphylococcus aureus, Micrococcus luteus, Pseudomonas putida F1, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans*) on measurement range 2000 – 500 $cm^{-1}$

## CONCLUSIONS

- From the research done on the microorganisms Staphylococcus aureus, Micrococcus luteus, Pseudomonas putida F1, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Candida albicans and concluded that they can differentiate using FT-IR technique.
- Differences between organisms are observed between 1500 - 1300 $cm^{-1}$ wavelength area called "finger print". The most significant area of study is in the range 1200-800 cm-1 which can differentiate microorganisms raise or lower absorbance values with different values.
- FT - IR technique can be used for rapid identification of microorganisms. Being only need a small amount of cultureof 24 hours and 5 minutes to read to the spectrophotometer IR light. Time to get a result is much lower than using conventional methods of identification.
- FT - IR Identification techniques is a fast and accurate identification of microorganisms (organisms composition differs, molecular composition in generals is different and FT-IR spectra of them will be different) with shortening of identification time.

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