Differentiation of Mixed Bacteria in the Generic Level Using Infrared Spectroscopy and Multivariate Analysis

Research proposal:

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Abstract:

Bacteria are considered as a domain or kingdom of prokaryotic micro-organism and they are divided into genera, species and isolates by their similarities. Bacteria and the infections that they can cause are among the primary causes of serious health problems worldwide, which can lead to high rates of mortality, particularly in undeveloped countries. Bacteria cause deadly diseases such as tuberculosis and cholera, as well as many other bacterial infections that can be prevented or cured by antibiotics. In order to help the physician choose antibiotics that are most suitable to prevent or reduce the severity of a human infection, it is important to detect all types of bacteria in a given sample taken from that infection. Today, it takes between 24-48 hours to classify the type of the infected bacterium. This time is crucial in many cases for saving the life of the patient. Many studies have been carried out and showed that it is possible to differentiate between different bacterial samples in the species level based on their infrared absorption spectra of the measured samples.

In the present study we will examine the potential of Fourier Transformed Infrared (FTIR) spectroscopy for accurate identification and differentiation of mixed bacteria samples in a time span of a few minutes. Multivariate analysis like Principal components analysis (PCA) followed by linear discriminant analysis (LDA) as a linear classifier will be performed on the measured spectra.

Goals:

Our goal is to predict the type of the bacterium using FTIR-ATR and multivariate analysis. The method is based on the infrared absorption spectra of the bacteria. We will start by performing several preprocessing steps, including growing the bacteria in the lab and leaching them from the medium. We will continue by measuring and characterizing the spectrum of the bacteria in general and of each bacterium separately. Finally we will mix different species of bacteria in different concentrations and try to predict the type of the bacteria using multivariate analysis of the features spectrum.

Literature Review:

Bacteria are considered as a domain or kingdom of prokaryotic micro-organism (organism that lacks a distinct nucleus and other organelles due to the absence of internal membranes) [1] and were among the first life forms to appear on earth. Some bacteria can cause diseases in humans, animals, or plants and for this reason their classification in the lab is of great importance. Today few classification methods of bacteria exist, for example: K. Rudi et al. [2] suggest rapid identification and classification of bacteria by 16S rDNA restriction fragment melting curve analyses
RFMCA, which is considered as an accurate method, but not suitable for routine applications in the industry. R. Rulaningtyas et al. [3] suggest automatic classification of Tuberculosis bacteria using Neural Network, by using an image of the sample. D. Parisi et al. [4] suggest analysis and classification of bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, which is a feasibility and rapid technique for analysis of bacteria, but required highly standardized instrumental analytical performance and bacterial culture conditions in order to achieve useful information. B. Rajwa et al. [5] suggest morphotypic analysis and classification of bacteria and bacterial colonies using laser light-scattering, pattern recognition, and machine-learning system, which can potentially recognize and classify bacterial species or strains for which there are no available antibodies or genetic markers, but required reliance on supervised learning and training libraries. A. Suchwalko et al. [6] suggest computer-based classification of bacteria species by analysis of their colonies Fresnel diffraction patterns. The method obtained classification error smaller than 10%, but is very sensitive for small speckles in the sample. FTIR spectroscopy is being more frequently examined for its potential as a diagnostic tool in medicine and biology because it is a reagent-free and rapid method. In the past few decades many researchers have demonstrated that IR spectroscopy is a useful method for bacterial identification [7-15]. FTIR spectroscopy has shown encouraging trends in the field of medicine. Various bimolecular components of the cell give rise to a characteristic spectrum that is rich in structural and functional aspects [16,17]. Previous results from the Medical Physics laboratory at the Department of Physics at BGU have shown that IR spectroscopy can detect early development of diseases or cell transformation at a stage when even the morphology is still considered normal [18,19]. For microbiological identification, both supervised and unsupervised methods can be used in the chemometric techniques [20-24]. Naumann et al. have shown that FTIR absorption spectra are highly specific fingerprints of microbial cells and that by using IR spectroscopy it is possible to differentiate between bacterial cells even in the isolate level [1,25].

In this study we will use the FTIR-ATR spectrometer and multivariate analysis in order to classify different species of mixed and pure samples of bacteria.

**Methods:**

We will start with growing different species of bacteria in the lab using an incubator in order to accelerate the process. Afterwards, leaching the bacteria from the medium will be done when a homogenous solution will be received, then we will continue by mixing different species of bacterium in different concentrations. Our next step will be to measure, using the FTIR-ATR system, the absorption spectrum of each bacterium separately and each mixed bacteria. Using the FTIR-ATR computer program, spectral processing including: Bisecting, Baseline correction and Normalization will be done on the absorption signal. Finally, by using multivariate
analysis including: PCA (principle component analysis), LDA (linear discriminant analysis) and Cluster analysis on the absorption spectrum, we will try to predict the type of the bacteria.

It is very important to emphasize that pre-processing, measuring, spectral processing and multivariate analysis will be done simultaneously.
References:


