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Identification of gram-negative and gram-positive bacteria by fluorescence studies JONATHAN DEMCHAK, JOSEPH CALABRESE, MARIAN TZOLOV, Lock Haven University of PA — Several type strains of bacteria including *Vibrio fischeri*, *Azotobacter vinelandii*, *Enterobacter cloacae*, and *Corynebacterium xerosis*, were cultured in the laboratory following standard diagnostic protocol based on their individual metabolic strategies. The bacterial cultures were not further treated and they were studied in their pristine state (pure culture - axenic). The fluorescent studies were applied using a continuous wave and a pulsed excitation light sources. Emission and excitation spectra were recorded for the continuous wave excitation and they all show similar spectral features with the exception of the gram positive bacteria showing vibronic structures. The vibrational modes involved in these vibronic bands have energy typical for carbon-carbon vibrations. The fluorescence is quenched in addition of water, even a very thin layer, which confirms that the observed spectral features originate from the outer parts of the bacteria. These results allow to conclude that the fluorescence spectroscopy can be used as a method for studying the membranes of the bacteria and eventually to discriminate between gram positive and gram negative bacteria. The pulsed experiments show that the fluorescence lifetime is in the sub-microsecond range. The results indicate that the observed spectra are superposition of the emission with different lifetimes.

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