

Variations in FTIR spectra between opaque and transparent phenotypic variants of *Streptococcus pneumoniae*

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Abstract

Streptococcus pneumoniae undergoes spontaneous and reversible phase variation in colony morphology, which is apparent as differences in colony opacity on transparent agar surfaces. These differences appear to be relevant to the pathogenesis of pneumococcal diseases. FTIR spectroscopy has shown remarkable ability in distinguishing between bacterial species, when used in tandem with mathematical methods like cluster analysis, principal component analysis or linear discriminant analysis. It can be used to identify bacterial colony structures or classify till intraspecific levels in a short time span. In the present work, Fourier transform infrared microspectroscopy (FTIR-MSP) analysis was carried out on opaque and transparent *Streptococcus pneumoniae* isolates, to understand the manifestation of the biochemical differences. In parallel, colony morphology was assessed with a stereo zoom microscope with oblique transmitted illumination. In general, capsular absorbance of the opaque phenotype was higher than in the transparent variant in the region 900-1185 cm^{-1} , where carbohydrates absorbance dominates except p2 which lacked a capsule. The opaque and transparent forms differed in other biochemical components like lipids and proteins, which was elucidated from the spectral data. The unsupervised cluster analysis of the samples showed differences not only in the carbohydrate content but also in the region of amide I, II and 2800-3100 cm^{-1} where proteins and lipids absorb respectively. The present work shows the potential of utilizing FTIR spectroscopy for distinction between the two phenotypic variants of *Streptococcus pneumoniae* based on the different amounts of capsule.

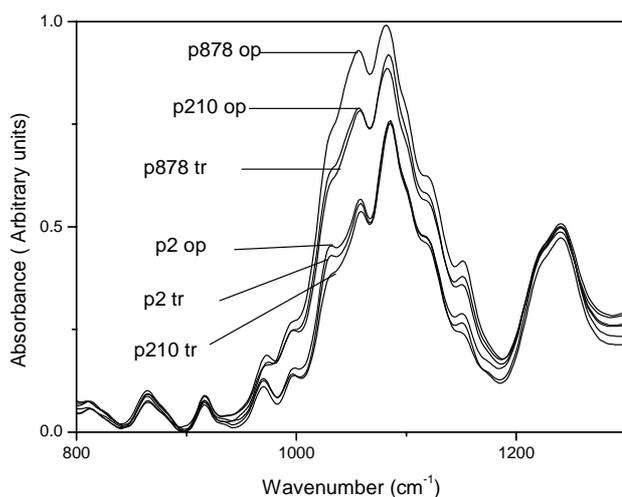


Fig. 1. Variations in the absorbance in the region 800-1300 cm^{-1} of various isolates of *S. pneumoniae* indicating the differences between opaque and transparent forms after normalization to the Amide I band at $\sim 1655 \text{ cm}^{-1}$: op (opaque) and tr (transparent) forms of the isolates.

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