Taxonomic Value of Infrared Spectra

D. N. WRIGHT* AND W. R. LOCKHART

Department of Bacteriology, Iowa State University, Ames, Iowa

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Efforts to demonstrate species or generic relationships among bacteria through the use of infrared (IR) spectra of whole cells (Norris, J. Hyg. 57:326, 1959) have been increasing despite frequent cautions (Kenner et al., J. Bacteriol. 75:16, 1958) that such procedures are fraught with experimental pitfalls. Many of the reports concerning bacterial identification by this technique are favorable and indicate a general acceptance of the method.

While investigating the balanced growth of Escherichia coli K-12 (Wright, Ph.D. Thesis, Iowa State University, Ames, 1964), IR spectra were obtained from cells grown in a defined medium with either the carbon or nitrogen source as the limiting substrate, and at either a high (0.5 generation per hr) or a low (0.1 generation per hr) growth rate. The medium contained: 0.7% KH2PO4, 0.3% K2HPO4, 0.01% MgSO4·7H2O in deionized water, to which was added 400 µg of (NH4)2SO4 per ml, and either 500 µg of glucose per ml (for carbon-limited cultures) or 4,000 µg of glucose per ml (for nitrogen-limited cultures). Rate of growth was controlled by the dilution rate in continuous culture. Pressed KBr discs were prepared with cells from each culture, and the absorption spectra between 850 and 1,820 cm⁻¹ were determined in a Beckman IR-7 spectrophotometer.

IR spectra of genetically identical cells, with all environmental factors controlled and differing only in their rate of growth or limiting substrate, show considerably greater differences (Fig. 1) than those previously reported as significant in differentiating strains of a species (Scopes, J. Gen. Microbiol. 28:69, 1952) or the species of a genus (Levine et al., J. Infect. Dis. 96:193, 1955).

If the IR patterns (Fig. 1) are analyzed by means of base line ratios (Wright, Ind. Eng. Chem. Anal. Ed. 13:1, 1941), it is apparent that the spectra are indeed different, suggesting that each cell type produces a characteristic pattern dependent both on the growth-limiting substrate and the rate of growth. The quantitative relationships among the various absorption bands were found by dividing their absorbancy by the absorbancy at 1,530 cm⁻¹. The ratios from the most prominent absorption bands indicate a direct correlation between growth rate and the cellular content of nucleic acid and polysaccharide (Table 1). These results suggest that IR used in association with physiological and biochemical studies (Wright and Lockhart, unpublished data) may be much more useful for extended examination of one species than for identification or classification.

A careful examination of reported IR spectra reveals that there is general similarity in the spectra from bacteria of several genera, although Thomas and Greenstreet (Spectrochim. Acta 6:302, 1955) were able to distinguish between several major taxa. Frequently, the separation of species within a genus has been based on differences no greater than those found with a single species and by varying the environment, and the separation of strains within a species is even more tenuous. The fact that the IR-absorption spectrum of a single strain of bacteria is altered by a change in growth rate may be reason to suspect work where this parameter has not been considered. Conclusions thus far reported are based upon differences in IR absorption spectra of Escherichia coli K-12 between 850 and 1,820 cm⁻¹. (A) Nitrogen-limited cultures grown at 0.5 generation per hr, (B) nitrogen-limited cultures grown at 0.1 generation per hr, (C) carbon-limited cultures grown at 0.5 generation per hr, and (D) carbon-limited cultures grown at 0.1 generation per hr. The four ordinate axes are drawn to the same scale, but with different origins to avoid superimposition of the spectral tracings.
Table 1. Ratio of absorbancy of three IR spectral bands of Escherichia coli to the absorption band at 1,530 cm⁻¹

<table>
<thead>
<tr>
<th>Limiting substrate</th>
<th>Growth rate (generations per hr)</th>
<th>1,240/1,530*</th>
<th>1,130/1,530†</th>
<th>1,075/1,530‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen . . . . .</td>
<td>0.5</td>
<td>0.652</td>
<td>0.418</td>
<td>0.738</td>
</tr>
<tr>
<td>Nitrogen . . . . .</td>
<td>0.1</td>
<td>0.505</td>
<td>0.535</td>
<td>0.518</td>
</tr>
<tr>
<td>Carbon . . . . . .</td>
<td>0.5</td>
<td>0.627</td>
<td>0.403</td>
<td>0.707</td>
</tr>
<tr>
<td>Carbon . . . . . .</td>
<td>0.1</td>
<td>0.523</td>
<td>0.377</td>
<td>0.553</td>
</tr>
</tbody>
</table>

* Representative of nucleic acid.
† Representative of carbohydrate.
‡ Representative of polysaccharide.

Spectra between species or strains within a genus are possibly overoptimistic, and, except in some rather special circumstances, such differentiation probably cannot be made accurately, or with any degree of reproducibility. It is therefore suggested that IR-absorption data be interpreted with great caution when used for taxonomic purposes, and that extremely precise metabolic control is necessary if meaningful IR absorption results are to be obtained in terms of taxonomic relationships.

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