

## FT-IR Spectroscopic Characteristics of Differently Cultivated *Escherichia coli*

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### Abstract

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FT-IR spectra were recorded of *Escherichia coli* cell mass with the aim of obtaining spectral traits possibly useful in a rapid detection and characterisation of this indicator bacterium. A well differentiated spectrum was obtained from the cell mass harvested in a stationary phase of growth, e.g., after 24 h, from a minimum nutrient broth. The cell mass, harvested either earlier or grown in nutrient solutions which contained an enhanced carbon or nitrogen concentrations delivered somewhat different IR spectra, apparently due to a higher content of nucleic acid components as related to other structural constituents of bacterial cells. Consequently, the FT-IR spectra of *E. coli*, although rather rapidly to collect, seem only capable of delivering useful and reproducible information if the cell mass is obtained under standardised cultural conditions.

**Keywords:** *Escherichia coli*; FT-IR spectroscopy; cell mass traits

*Escherichia coli* is a non-sporeforming, gram-negative bacterium, about  $2\text{--}6 \times 1.0\text{--}1.5 \mu\text{m}$  in size. It belongs to the family *Enterobacteriaceae*, typically inhabits mammalian intestine, and is therefore widely used as an indicator of fecal pollution of water and different foodstuffs. Usually, *E. coli* demonstrates maximum growth at  $37\text{--}39^\circ\text{C}$ , but in fact this bacterium is capable of growing over a span of more than  $40^\circ\text{C}$ , from about  $8^\circ\text{C}$  to  $50^\circ\text{C}$  (KÁŠ 1966; NEIDHARDT 1995; LEADBETTER 2002). In sand or soil, *E. coli* can utilise simple organic substrates such as glucose under the release of  $\text{CO}_2$ , but in liquid cultures the respiratory activity was found to be markedly enhanced also in the presence of alkanes such as dodecane, tridecane, and tetradecane (KÁŠ 1966; MORISAKI 1984). The

latter author (MORISAKI 1982) has demonstrated the generation of electric current on the surface of *E. coli* cells, and perhaps this capacity and the surface display of various bioactive molecules can be made responsible for a strong accumulation of bivalent heavy metals such as  $\text{Cd}^{2+}$  by this bacterium (SOUSA *et al.* 1998; KOTRBA *et al.* 1999). Even more important, different strains of *E. coli* have been associated with enteropathogenic, enterotoxigenic, enteroinvasive, and hemorrhagic human diseases (KELLY *et al.* 1985; MOE 2002). For all these reasons, but especially because *E. coli* is capable of indicating the presence of many other microbial or viral enteric pathogens, the detection and enumeration of this bacterium especially in water, sewage, and different foodstuffs represent

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an important hygienic standard (KÁŠ 1964; DUFOUR 1977; KOTT 1977; WHEATER *et al.* 1980). Numerous laboratory procedures have been developed for this purpose, the majority of which is rather indirect, i.e., based on some metabolic traits such as the capacity of *E. coli* either to utilise or not to different substrates, and on serotyping of the individual isolates (SMIBERT & KRIEG 1981; KELLY *et al.* 1985; EDBERG *et al.* 1991). Here, we undertake an attempt to characterise *E. coli* more directly by using infrared (IR) spectroscopy in order to determine structural traits of the entire cell mass. The same analytic techniques have been recognised as useful in many fields of biochemistry, biology, and medicine (PARKER 1971; NAUMANN 2000). In our early investigations, we obtained well differentiated IR spectra from a complex soil microbial biomass (FILIP 1978). Later on, we used the Fourier Transform-IR spectroscopy (FT-IR) for the characterisation of the cell mass of *Pseudomonas* spp., and *Bacillus subtilis* (FILIP & HERMANN 2001; FILIP *et al.* 2004).

#### MATERIAL AND METHODS

The tested strain of *E. coli* was obtained from the German Culture Collection of Microorganisms and Cell Cultures in Braunschweig. The freeze dried inoculum was cultivated at 30°C for 24 h in a Minimum Nutrient Broth (MNB) whose the composition had been described elsewhere (FILIP & HERMANN 2001). The cell mass was collected by centrifuging the cultures at 10 000 × g for 15 min subsequently, it was repeatedly washed with deionised water, centrifuged again and freeze dried prior to further use. Starving bacterial cells were also prepared. These were obtained after a freshly harvested cell mass was re-suspended in 50 ml deionised water and incubated at 30°C for 24 h on a shaker. In some experiments, the cell mass was harvested from MNB in different phases of the cultivation (between 3 h and 168 h) in order to determine the stability or variability of the IR traits in the dependence on the age of the *E. coli* culture. Furthermore, in some cultures (i) MNB was replaced by Luria-Bertani broth (Merck), (ii) in MNB glucose was replaced with fructose, (iii) ammonium chloride was replaced with through 10mM glutamate, or (iv) 40mM of glutamate served as both N and C sources.

For the FT-IR spectroscopy, the absorption/transmission technique was used (JOHNSTON

1991). The spectra were recorded of 2 mg of cell mass in 300 mg KBr (Uvasol® Merck, Darmstadt) discs which were pressed at 250 atm and dried under vacuum prior to scanning. The samples were examined in a Bruker IFS 48 FT-IR Spectrophotometer over the waverange 4000–400 cm<sup>-1</sup> (2.5–25 μm). Before and during the measurements, the IR-chamber of the device was purged with a pre-dried CO<sub>2</sub>-free air in order to prevent spectral interference which might be caused by free H<sub>2</sub>O or CO<sub>2</sub> molecules. A repeated scanning of the individual discs did not affect the appearance of the respective spectra.

#### RESULTS AND DISCUSSION

There is no doubt that *E. coli* proliferates best under copious nutritional conditions. Nevertheless, it can also survive under starvation which, however, evokes changes in the physiological activity of the stressed cells (KOCH 1979; KOLTER 1995). As shown in Figure 1, *E. coli* cell mass harvested from a standard culture, i.e., after 24 h cultivation in MNB, as well as that one obtained from starved cells developed both well differentiated but quite identical IR spectra. This feature indicates (i) that starvation did not affected the composition of

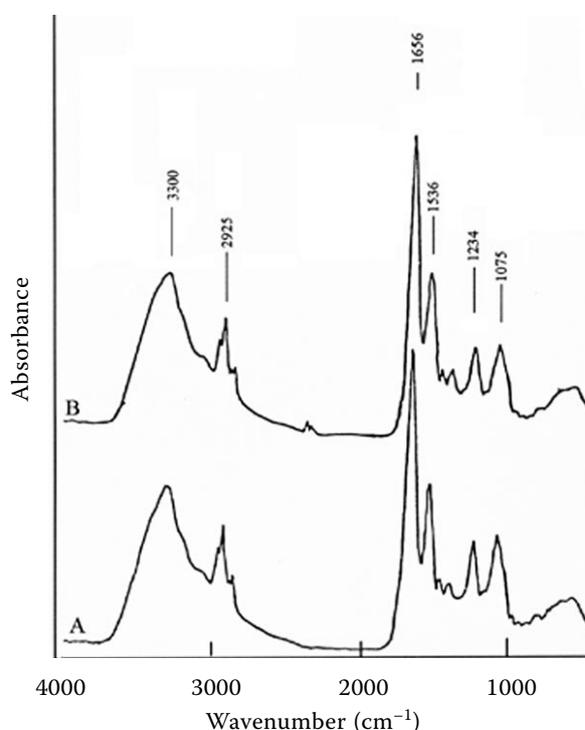


Figure 1. FT-IR spectra of *E. coli* cell mass harvested after 24 h cultivation (A), and of a starved cell mass (B)

Table 1. Infrared absorption bands of *Escherichia coli*

Band (cm <sup>-1</sup> )	Possible assignments*
3300–3000	NH <sub>2</sub> stretching, e.g., in adenine, cytosine, guanine; H-bonded OH groups
2960–2850	C-H stretching in aliphatics of cell walls (fatty acids, carbohydrates)
1660–1650	NH <sub>2</sub> bending, C=O, C=N stretching (amide I band)
1540–1535	Amide II band
1460–1455	C-H deformations of CH <sub>2</sub> or CH <sub>3</sub> groups in aliphatics
1396–1389	C-H bending, -CH <sub>3</sub> stretch in fatty acids
1240–1234	Amide III band
1150–1030	C=O, P=O, P-O-C (P-O-P) asymmetric stretching (glycopeptides, ribose, aliphatic esters)
650–480	C-O-C, P-O-C bonding (phospholipids, RNA, aromatics)

\*Assignments after PARKER (1971), NAUMANN (2000), and others

cell mass, and indirectly, (ii) that our treatment of the cell mass prior to IR scanning was effective enough for removing any component of the nutrient solution possibly adhering on the bacterial cells.

The assignments of the individual IR-absorption bands are listed in Table 1. A dominant absorption that is often attributed to O-H stretching of hydroxyl groups was recorded at 3300 cm<sup>-1</sup>. Because the free water band absorbing at 1613 cm<sup>-1</sup> could not be detected (apparently due to a sample pre-treatment as mentioned in the previous section), the strong and broad absorption band (3300 cm<sup>-1</sup>) should be attributed mainly to N-H stretching vibrations of nucleic acids components such as adenine, guanine, and/or cytosine, and only in part to some OH groups possibly adsorbed on the nucleic acids molecules (PARKER 1971). Quite a few spectral peaks appeared in the typical C-H stretching region between 2960–2850 cm<sup>-1</sup>. Here, some cell wall constituents usually found in Gram-negative bacteria such as lipids, lipoproteins, and polysaccharides typically absorb (ROSE 1965). The IR absorption of cell proteins delivered several amide-related bands. These dominated at 1656 cm<sup>-1</sup> (amide I region), 1536 cm<sup>-1</sup> (amide II), and 1234 cm<sup>-1</sup> (amide III). More specifically,  $\alpha$ -helical structures ( $\sim$  1655 cm<sup>-1</sup>) and  $\beta$ -pleated sheet structures ( $\sim$  1637 cm<sup>-1</sup>) in microbial amides absorb at these frequencies according to NAUMANN (2000). Stretching vibrations of -CH<sub>2</sub> and -CH<sub>3</sub> functional groups in some aliphatic compounds, e.g. fatty acids, could be detected at 1454 cm<sup>-1</sup> and 1393 cm<sup>-1</sup>. Carbonyl groups in cell wall related glycopeptides, as well as P=O stretching, and

P-O-C (P-O-P) bonds in phospholipids and esters should be considered responsible for the strong absorption at 1080 cm<sup>-1</sup>. The IR region between 900 and 600 cm<sup>-1</sup> exhibited some rather broad but weak spectral features which might belong to aromatic ring vibrations. According to NAUMANN (2000), an exact assignment of this part of the IR spectrum is hardly to achieve.

Figure 2 shows the growth curve obtained with *E. coli* cultivated in MNB solution. A short lag-phase lasted for 3 h was followed by a strong exponential growth of the bacterial population until maximum cell density was reached after 24 h. Then, in the stationary phase which lasted for 2 days, the cell density oscillated at a high level of  $\sim$ 10<sup>9</sup> ml<sup>-1</sup> until day 3 (72 h) of the cultivation. Thereafter, the death phase followed in the *E. coli*

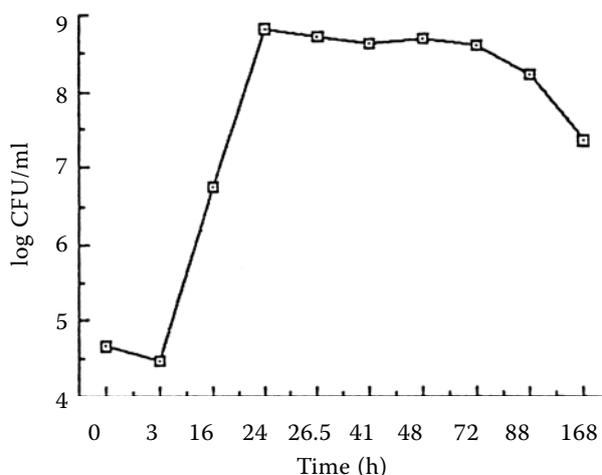


Figure 2. Growth curve of *E. coli* cultivated in a minimum nutrient broth

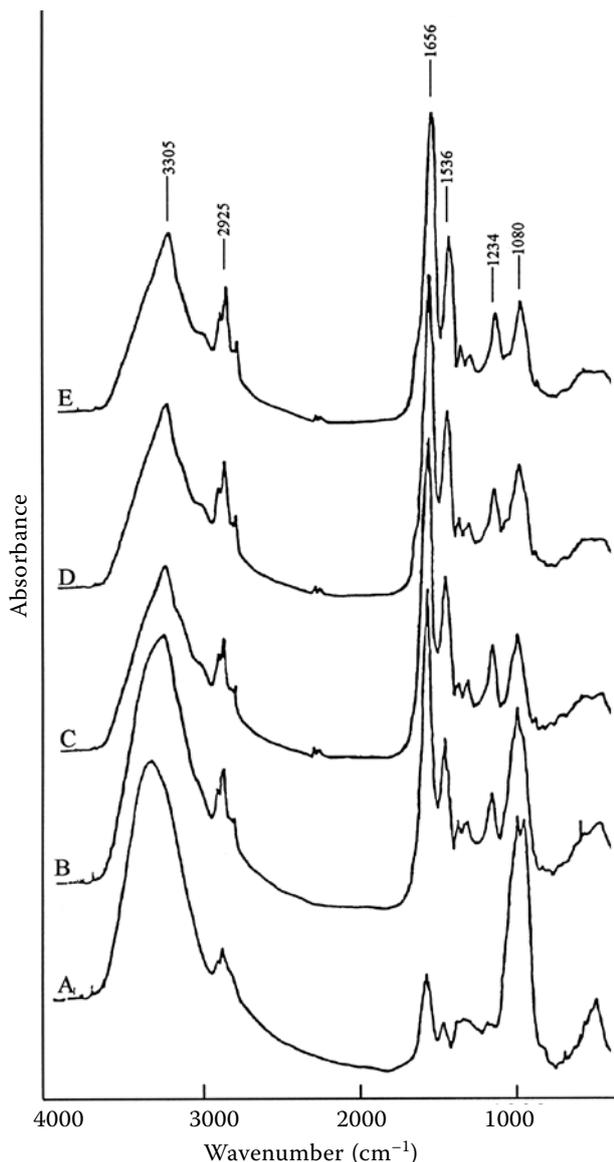


Figure 3. FT-IR spectra of *E. coli* cell mass harvested after (A) 3h, (B) 16 h, (C) 41 h, and (D) 168 h from a minimum nutrient broth

population and continued until the cultivation was terminated after 168 hours.

In Figure 3, the FT-IR spectra of differently aged *E. coli* cell mass are shown. There is clear evidence that the composition of the cell mass harvested in early phases of growth (Figure 3 A–B) differed strongly from the older ones (Figure 3 C–E). The spectra of the cell mass harvested after 41, 72, and 168 h not only resembled one another but also those shown in Figure 1. This indicates a high degree of stability in the composition of *E. coli* cell mass after 24 h of cultivation, which remained unchanged also in the stationary growth phase. Contrary to this,

the cell mass harvested from a 3 h culture, i.e., at the end of the lag-phase, delivered an IR spectrum with a strong absorption dominance at  $3300\text{ cm}^{-1}$ . A second very strong peak demonstrated the absorbance splitting at 1150, 1080, and  $1030\text{ cm}^{-1}$  frequencies. According to GRAY and WILKINSON (1965), phosphatidyl-ethanolamines characteristically absorb at  $1030\text{ cm}^{-1}$ , and after RANDLE *et al.* (1969), the content of this compound in the cell mass of *E. coli* diminishes in the stationary phase of growth. This seems to correspond well with the decrease of the respective IR absorption in our spectra (Figure 3 C–E). At  $540\text{ cm}^{-1}$ , a rather strong absorption peak appeared in the 3 h cell mass, while the absorptions at  $2960\text{--}2800\text{ cm}^{-1}$ ,  $1656\text{ cm}^{-1}$ , and  $1540\text{ cm}^{-1}$  were much less developed in comparison with the spectra obtained from the older cell mass. Again, these features indicate a strong participation of nucleic acid components in the young cell mass in relation to cell-protein components (PARKER 1971). According to the differences in the values of the ratio calculated from the relative intensity (a base to peak high) of the absorption bands at  $1656\text{ cm}^{-1}$  (amide I), and  $1080\text{ cm}^{-1}$  (P=O stretching), i.e., 0.44 after 3 h, 1.60 after 16 h, and 2.60 after 41 h of growth, respectively, a relative increase can be assumed of nitrogenous structures in the *E. coli* cell mass in the cultivation. The nucleic acid related absorption at  $540\text{ cm}^{-1}$  became hardly detectable in the cell mass harvested in a later stationary phase of growth (Figure 3 D–E). The ratio calculated from the absorptions at  $2925\text{ cm}^{-1}$  (C-H stretching in aliphatic compounds), and  $1656\text{ cm}^{-1}$ , i.e., 1.62 after 3 h and 0.42 after 72 h, indicates an increased participation of aliphatic cell wall components in the older cell mass.

From Figure 4 can be recognised that the IR spectra of the *E. coli* became evidently affected by the composition of the nutrient broth. The cell mass from the cultures containing glutamate and high concentrations (40 mmol) of either glucose or fructose (Figure 4 C–E) demonstrates a very strong absorption at  $3300\text{ cm}^{-1}$ , and on the contrary, a weak one at  $1234\text{ cm}^{-1}$ . An absorption triplet appeared at 1150, 1080, and  $1030\text{ cm}^{-1}$  in the cell mass harvested from cultures enriched in glucose or fructose. In this respects, the IR spectra resembled those of the cell mass harvested in the early growth phase from MNB (Figure 3 A–B). Apparently, copious nutrient conditions allowed an extensive growth of *E. coli* and resulted in higher

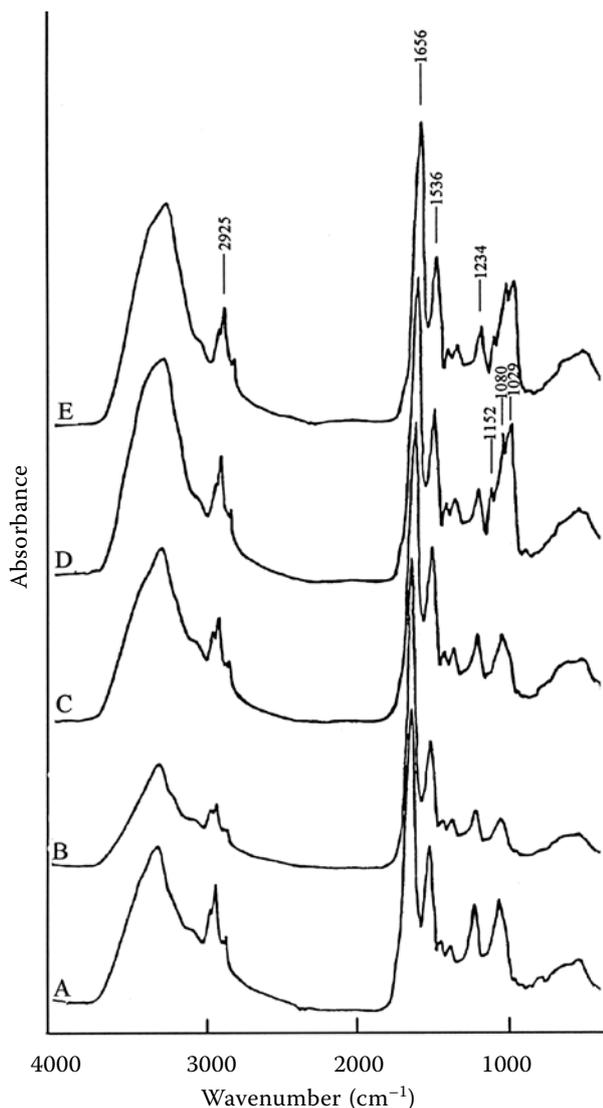


Figure 4. FT-IR spectra of *E. coli* cultivated 24 h in (A) minimum nutrient broth, (B) Luria-Bertani broth, (C) minimum nutrient broth less N + 40 mmol glutamate, (D) minimum nutrient broth less N + 10 mmol glutamate + 40 mmol glucose, (E) minimum nutrient broth + 40 mmol fructose

counts of young cells with an enhanced proportion of nucleic acids whose structural components specifically affected the IR spectra. The Luria-Bertani broth seemed not to be optimum for the *E. coli* growth. The IR spectrum of the cell mass harvested from this culture medium (Figure 4B) resembled basically that obtained from MNB but the intensity of the peaks was evidently lower.

In comparison with the results of our previous IR investigations on microorganisms (FILIP 1978; FILIP & HERMANN 2001), the spectra of the *E. coli* cell mass clearly differed from those

of a complex soil microbial biomass. However, a close resemblance to the spectra obtained from *Pseudomonas* spp. apparently exists, thus further investigation seems necessary to confirm or to deny the usefulness of the FT-IR spectroscopy for the characterisation of individual gram-negative bacteria in samples of different origin. Basically, however, to obtain well stabilised FT-IR spectra, the cell mass harvested from a standard nutrient broth, and in the stationary phase of bacterial growth, should be used for the scanning.

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