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A comparative study of fresh and lyophilised biomass of the bacterium *Enterobacter cloacae* K-7 by vibrational spectroscopy techniques

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A comparative analysis of Raman spectra of fresh (separated by centrifugation and washed from the culture medium; without drying) and lyophilised biomass samples of planktonic culture of the bacterium *Enterobacter cloacae* K-7, as well as of a Fourier transform infrared (FTIR) spectrum of the lyophilised biomass, was performed. It has been shown that for fresh biomass, the presence of a significant amount of water in the sample does not lead to masking or shifting the main bands of the functional groups of biomacromolecules characteristic of bacteria in the case of Raman spectra, compared to lyophilised *E. cloacae* cells. For the latter, both the Raman and FTIR spectra also contain vibrational bands of the main functional groups typical of bacteria. The results obtained indicate the possibility of using both methods of sample preparation of bacterial biomass samples for general comparative analysis of their biomacromolecular composition by Raman spectroscopy and, in the case of lyophilised biomass, by FTIR spectroscopy.

Keywords: bacterial biomass, Raman spectroscopy, FTIR spectroscopy, lyophilization, *Enterobacter cloacae*.

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Introduction

Vibrational spectroscopy techniques (primarily infrared (IR) spectroscopy and Raman spectroscopy) in the last decades have shown a steady growth of their use in various fields of microbiology [1–4], including their joint use due to the complementarity of the data obtained [5–8]. This is largely due to the relative simplicity of sample preparation and measurements, while it is possible to obtain information about the total biomacromolecular composition of samples at the level of molecular functional groups (by vibration bands of groups of atoms). Nevertheless, the downside of such simplicity is the requirement of strict adherence to the basic methodological rules for obtaining informative spectra reflecting the actual state of the studied microbiological objects. In many cases, rich information contained in vibrational spectra makes their interpretation difficult. In view of this, methodological studies focused on these techniques are still relevant (for example, [9–13]).

In this study, a comparative analysis of Raman spectra of fresh biomass (separated by centrifugation and washed from the culture medium; without drying) and the lyophilised biomass of the planktonic culture of *Enterobacter cloacae* K-7 obtained from it, as well as of a Fourier transform infrared (FTIR) spectrum of the latter, was carried out. The interest in this studied object is caused by the fact that many bacteria of the genus *Enterobacter* have a phytostimulating effect on higher plants [14–16], have other useful properties from the agricultural biotechnology standpoint and are used in biofertilisers [17]. Apart from it, *E. cloacae* itself has some additional useful properties [14,18–20]. Lyophilisation (freezing of cell biomass followed by drying in vacuum) is one of the main industrial and laboratory methods for obtaining dry microbial preparations for their long-term storage [21,22]. It is important to note that at the same time, a significant part of the cells remain viable, passing into a dormant (metabolically inactive) state, which is widely used in various fields of microbial biotechnology [22–24]. It should be stressed that soil bacteria also encounter cyclic drying processes with repeated moistening in the natural environment while keeping their viability [25]. For the practical use of dry bacterial preparations, in particular as part of biofertilisers, their safety and storage efficiency are critical, which is paid special attention [26,27].

Materials and research methods

Preparation of bacterial culture

Culture of *E. cloacae* K-7 was obtained from the Collection of Rhizosphere Microorganisms maintained at IBPPM RAS, Saratov, Russia (accessed 20.11.2025; https://collection.ibppm.ru/collection/enterobacter/enterobacter-cloacae/); the strain was isolated from Jerusalem artichoke rhizoplane (*Helianthus tuberosus* L.) [20]. The preculture was grown under standard conditions, after which the culture was raised to the end of the logarithmic growth phase (18 h) on the same saline malate medium as previously indicated [13], separated by centrifugation, washed twice with saline solution (0.85% NaCl); part of the culture was dried by lyophilisation.

Measurement of Raman spectra

To obtain the Raman spectra of a fresh biomass, its preparation was used after centrifugation and washing (without drying); the preparation was stored in the refrigerator for maximum one day before measurements; prior to the measurements, the sample was placed in the holder (see below) and stored at room temperature for half an hour. The lyophilised biomass needed to get the Raman spectra was mixed with KBr (reagent for spectral analysis) in a ratio of 30 mg of biomass +70 mg KBr (to avoid decomposition of the sample exposed to a laser beam), ground for 5 min in an agate mortar until homogeneous and transferred to a sample holder — a glass tube (inner diameter 3 mm, wall thickness 1.5 mm). The measurements were carried out as described earlier [28] using an RFS-100 Fourier transform Raman spectrometer („Bruker", USA; laser with an excitation wavelength of 1064 nm, power at the sample ~ 0.1 W, spectrum accumulation time ~ 3 h, resolution 2 cm^{-1}; the instrument was controlled and the baseline correction of the spectra was performed using the OPUS Spectroscopy Software (USA), supplied with the spectrometer).

Measurement of FTIR spectra

To measure an FTIR spectrum, ~ 2 mg of lyophilised biomass of *E. cloacae* K-7 was suspended in 0.200 mL of distilled water, 0.100 mL of the obtained suspension was applied on a flat surface of a ZnSe glass disc (CVD-ZnSe, „R'AIN Optics", Dzerzhinsk, Russia; diameter 2.5 cm, thickness 2 mm) and dried at 45°C for 24 hours (a fairly uniform thin film was formed, tightly fitting to the ZnSe surface). A Nicolet 6700 FTIR spectrometer („Thermo Electron Corporation", USA) with a resolution of 4 cm^{-1} was used; other measurement parameters were described earlier [12].

Results and discussion

In the Raman spectrum of fresh biomass (Fig. 1, *a*), the presence should be noted of an intense, strongly broadened typical band of stretching vibrations of OH groups of water present in the sample, bound by strong hydrogen bonds, ν(O−H) with a maximum at about 3210 cm^{-1}. Some contribution of a broadened weak band corresponding to scissoring (deformational) vibrations of H−O−H (about 1640 cm^{-1}) is noticeable when comparing the intensity ratio of neighbouring biomass bands (at ~ 1663 and ~ 1450 cm^{-1}; see below) in Fig. 1, *a* and 1, *b*. With the exception of these differences, both Raman spectra are dominated by a composite band with a maximum at ~ 2934 cm^{-1}, which includes the most intense Raman bands of stretching vibrations ν(C−H) of various aliphatic groups (in the range $\sim 3000-2800$ cm^{-1}; these bands are weaker in IR spectra; Fig. 2). Among the major ones we may highlight the bands of cellular

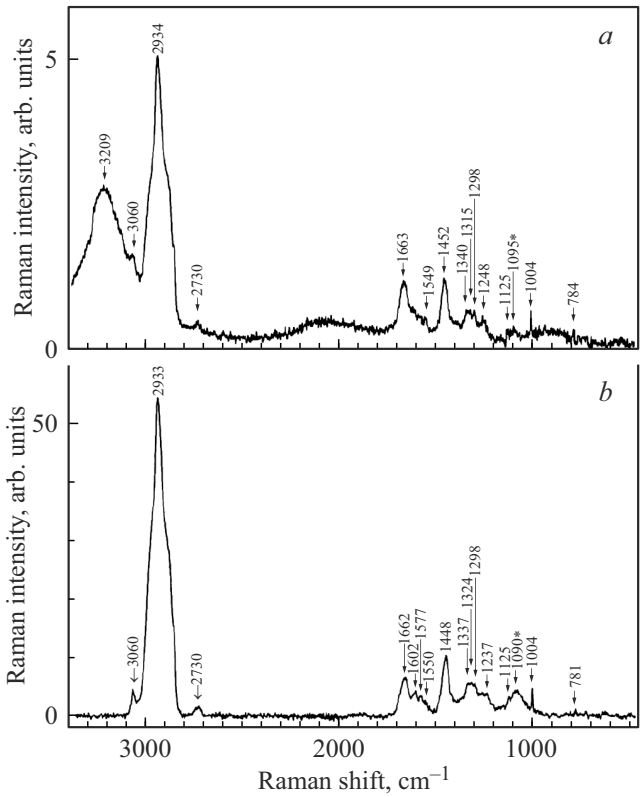

Figure 1. Raman spectra of fresh (*a*) and lyophilised (*b*) biomass of *Enterobacter cloacae* K-7. (* on the spectra denotes a wide region of glass absorption (about 1090 cm^{-1}) of the test tube where the sample was placed).

proteins — amide I (weaker in Raman spectra wide bands around 1663 cm^{-1} overlapped with an absorbance ν(C=C) of the unsaturated fatty-acid residues), amide II (~ 1550 cm^{-1} weak in Raman spectra and overlapped with the band ν(C=C) of indole nucleus of tryptophan) and amide III (weak; $\sim 1250-1240$ cm^{-1}); bands of deformational vibrations δ(C−H) of aliphatic groups (at ~ 1450 and $\sim 1340-1315$ cm^{-1}), DRA/RNA bands (at ~ 1125 and $784-781$ cm^{-1}), as well as a characteristic narrow band of planar deformational (breathing) vibrations of benzene cycles of phenylalanine and tryptophan around 1004 cm^{-1} [3,5,6,28].

When comparing the data in Fig. 1, *a, b*, it is obvious that the presence of a significant amount of water in fresh biomass does not mask or shift the main vibrational bands of functional groups of biomacromolecules specific for Raman spectra of bacteria as compared to the lyophilised cells of *E. cloacae*. In both spectra in Fig. 1, *a* and 1, *b*, the maxima and relative intensities of the main bands specific for Raman spectra of gram-negative bacteria (see, for example, [3,6,9,28]) are close or coincide.

It should be emphasized that the use of potassium bromide (KBr) as an optically transparent substance in the sample preparation of lyophilised biomass for measuring Raman spectra (see subsection „*Measurement of Raman spectra*") had no noticeable effect on the position and

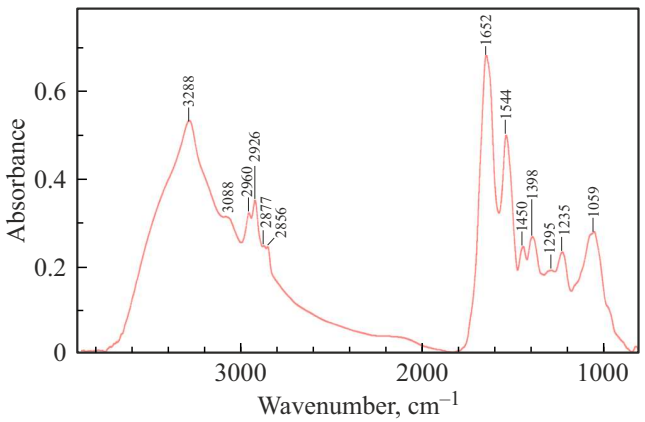

Figure 2. FTIR spectrum of lyophilised biomass of *Enterobacter cloacae* K-7.

relative intensity of the bands (Fig. 1, *b*). However, it is known that the use of the KBr polar matrix in the sample preparation of biological samples (including bacterial biomass) for IR spectroscopy can lead to a significant shift in the bands of polar functional groups [11]. In our case the absence of any noticeable effect of KBr in the Raman spectrum (Fig. 1, *b*) can be defined by the two major factors. Firstly, unlike IR spectra, the most intense in Raman spectra are the vibrational bands of weakly polar and nonpolar functional groups. Secondly, in this study, the biomass content in the mixture with KBr for measuring the Raman spectrum was 30 wt.%, whereas for IR spectroscopy using the KBr matrix, the sample content in the mixture is usually 1–2 wt.%. A much larger excess of KBr in the latter case (especially if the sample is pressed into pellets [11]) can evidently have a stronger effect on the polar bonds.

Analysing the Raman spectrum of fresh biomass (Fig. 1, *a*) obtained by centrifugation of an aqueous suspension of cells, we may assume that by the ratio of relative intensities of a wide strong band ν(O–H) of water with the maximum $\sim 3209\,\text{cm}^{-1}$ and the most intense band ν(C–H) of the bioorganic component (at $\sim 2934\,\text{cm}^{-1}$) the cell biomass in the aqueous suspension may be quantified (in other words, the density of the cell culture suspension). This option is related to the correlation of the relative band intensities of certain functional groups in Raman spectra with their total relative content in the studied sample. Nevertheless, such a quantitative assessment requires additional calibration measurements of Raman spectra of cell suspensions with certain density values (with known water content in the suspension).

The IR spectrum of lyophilised biomass of *E. cloacae* K-7 (Fig. 2) contains all the major bands characteristic of dried gram-negative bacteria (e.g., see [1,12,13,28]), namely, a wide band ν(O–H) of various biomolecules and water traces ($\sim 3600–2700\,\text{cm}^{-1}$) amid a backdrop of which the bands ν(N–H) of amides ($3288\,\text{cm}^{-1}$) and ν(C–H) of aliphatic groups are observed ($3000–2800\,\text{cm}^{-1}$), specific bands of cellular proteins — amide I ($1652\,\text{cm}^{-1}$), amide II ($1544\,\text{cm}^{-1}$) and amide III ($1235\,\text{cm}^{-1}$), deformational vibrations band δ(C–H) of the aliphatic groups ($1450\,\text{cm}^{-1}$), band of symmetric stretching vibrations $\nu_s(\text{COO}^-)$ of carboxylate groups at $\sim 1398\,\text{cm}^{-1}$ (the corresponding antisymmetric stretching band $\nu_{as}(\text{COO}^-)$ usually manifesting itself around $1580–1650\,\text{cm}^{-1}$ is masked by the protein bands of amide I and amide II), the absorption region of cellular polysaccharides ($\sim 1200–950\,\text{cm}^{-1}$).

The Raman spectra and IR spectrum of *E. cloacae* K-7 in the entire mid-IR range ($\sim 3500–500\,\text{cm}^{-1}$) (Figs. 1 and 2) are published, as far as we know, for the first time (in particular, the Raman spectrum for fresh biomass (without drying) obtained from the planktonic culture).

Raman spectra (for lasers with excitation wavelengths $\lambda = 785$ and 514.5 nm) of the air-dried strain *E. cloacae* A5149 in the range $1800–500\,\text{cm}^{-1}$ [29] and samples (in air-dry form or from agar substrates) of clinical isolates of *E. cloacae* have been published in the literature [30,31]. All of them show a similar position of the main bands of key functional groups of biomolecules. Also IR spectra of *E. cloacae* (ATCC 13047) within $1800–1000\,\text{cm}^{-1}$ [32], biofilms of *E. cloacae* 67E1 wild-type strain and some mutant strains by biofilm production were published [33].

For comparison, we note that according to FTIR spectra of even structurally more complex yeast cells (*Saccharomyces cerevisiae*), no differences were found in [34] between freshly prepared biomass (measurements were carried out on thin films quickly dried in vacuum) and lyophilised biomass, including the use of chemometric analysis of spectroscopic data, which is consistent with the data from this paper.

Conclusions

A comparative analysis of Raman spectra of the fresh (separated by centrifugation and washed from the culture medium; without drying) and lyophilised biomass samples of the planktonic culture of *Enterobacter cloacae* K-7, as well as the IR spectrum of the latter, showed that for fresh biomass, the presence of a significant amount of water in the sample in case of Raman spectra does not lead to any masking or shift of the major bands of functional groups of biomacromolecules characteristic of bacteria compared to the lyophilised cells. The results obtained indicate the possibility of using both methods of bacterial biomass sample preparation — wet biomass after centrifugation or after freeze-drying — for a general comparative analysis of their biomacromolecular composition by Raman spectroscopy, as well as (in case of lyophilised biomass) by IR spectroscopy.

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Compliance with ethical standards

This article does not contain any scientific results involving animals or humans used as the objects of study.

Conflict of interest

The authors declare that they have no conflict of interest.

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