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Instrumental analysis of bacterial cells using vibrational and emission Mössbauer spectroscopic techniques

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Abstract

In biosciences and biotechnology, the expanding application of physicochemical approaches using modern instrumental techniques is an efficient strategy to obtain valuable and often unique information at the molecular level. In this work, we applied a combination of vibrational (Fourier transform infrared (FTIR), FT-Raman) spectroscopic techniques, useful in overall structural and compositional analysis of bacterial cells of the rhizobacterium *Azospirillum brasilense*, with ^{57}Co emission Mössbauer spectroscopy (EMS) used for sensitive monitoring of metal binding and further transformations in live bacterial cells. The information obtained, together with ICP-MS analyses for metals taken up by the bacteria, is useful in analysing the impact of the environmental conditions (heavy metal stress) on the bacterial metabolism and some differences in the heavy metal stress-induced behaviour of non-endophytic (Sp7) and facultatively endophytic (Sp245) strains. The results show that, while both strains Sp7 and Sp245 take up noticeable and comparable amounts of heavy metals from the medium (0.12 and 0.13 mg Co, 0.48 and 0.44 mg Cu or 4.2 and 2.1 mg Zn per gram of dry biomass, respectively, at a metal concentration of 0.2 mM in the medium), their metabolic responses differ essentially. Whereas for strain Sp7 the FTIR measurements showed significant accumulation of polyhydroxyalkanoates as storage materials involved in stress endurance, strain Sp245 did not show any major changes in cellular composition. Nevertheless, EMS measurements showed rapid binding of cobalt(II) by live bacterial cells (chemically similar to metal binding by dead bacteria) and its further transformation in the live cells within an hour.

Keywords: Bacterial cells; Heavy metals; Metabolic processes; Spectroscopic analysis; Fourier transform infrared (FTIR) spectroscopy; Emission Mössbauer spectroscopy

1. Introduction

In diverse fields of biological sciences and biotechnology, the expanding application of physicochemical approaches using modern instrumental techniques is an efficient strategy to obtain valuable and often unique bioanalytical information at the molecular level. Various modifications of vibrational (Fourier transform infrared (FTIR), FT-Raman) spectroscopy have been extensively used for structural and compositional analysis of diverse biological materials [1–6]; in particular, as convenient and sensitive tools for monitoring both macroscopic changes in the cellular composition and fine structural rearrangements of cellular constituents [7–15].

The bioanalytical information jointly obtained by a combination of independent instrumental techniques may often be of advantage, especially when comparing the data on overall cellular metabolic changes (e.g., using vibrational spectroscopy) with analyses for microelements (e.g., trace metal uptake) and/or their chemical forms (speciation analysis). One of the extremely sensitive techniques is the emission variant of Mössbauer (nuclear gamma-resonance) spectroscopy (EMS) that has so far been relatively rarely used in bioscience [16]. Though nuclear analytical

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methods are generally not capable of speciation analysis [17], the EMS technique (not commonly regarded as analytical) can provide quantitative information on the content of chemical species of the Mössbauer-active element. The main limitation of EMS is that its use is restricted to a few such elements, the most convenient nuclide for EMS being the radioactive ^{57}Co isotope. Nevertheless, cobalt as a trace element with a broad range of biochemical functions is of paramount importance for many organisms [18,19]. It also attracts attention owing to biogeochemical problems related to bioleaching of the radioactive ^{60}Co isotope from disposal sites [20,21] facilitated by possible microbial dissimilatory reduction of Co^{III} oxide-containing minerals [22,23]. The EMS technique can readily be adapted for in situ studies, giving valuable quantitative information on the structure and rearrangements of cation-binding sites in biomolecules and metalloproteins [16,24,25].

In this work, we compared the results of FTIR spectroscopic analyses of whole cells of different bacterial strains under moderate heavy metal stress (induced by cobalt(II) as well as some other divalent cations (Cu, Zn) at submillimolar concentrations), with the data of emission Mössbauer spectroscopic monitoring of primary binding of [^{57}Co]-cobalt(II) by bacterial cells and its further transformations in live cells. The subject of this study was the plant-associated rhizobacterium *Azospirillum brasilense* that attracts attention owing to its phytostimulating potential [26], including its strain Sp245 which is known to be a facultative endophyte (capable of penetrating to and colonising the plant root interior), and non-endophytic strain Sp7 (colonising the root surface only), that occupy different ecological niches and show some differences in behaviour [27,28].

2. Experimental

2.1. Preparation of bacterial cultures

The bacteria *A. brasilense* (wild-type strains Sp7 and Sp245; the Collection of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia) were cultivated in a standard synthetic phosphate- and malate-containing medium as reported elsewhere [28,29], with 3 g l^{-1} NH_4Cl as a bound nitrogen source and 0.6% sodium malate as a carbon source (pH 6.9), under aeration by stirring on a rotary shaker. For FTIR spectroscopic measurements, along with using the standard medium (control), the bacteria were similarly cultured also in the same medium to which $CoCl_2$, $CuSO_4$ or $ZnSO_4$ had been added up to 0.2 mM.

2.2. Sample preparation and FTIR spectra acquisition

For FTIR in the transmission mode, cell samples were mixed with KBr (Merck) or, for diffuse reflectance infrared Fourier transform (DRIFT) measurements, used as dry finely ground powder in a Micro sampling cup (Spectra-Tech Inc., USA). FTIR studies were performed using a Perkin-Elmer (Model 2000) or (for DRIFT) a Nicolet spectrometer (model Magna-IR 560 E.S.P.) with a total of up to 100 scans (resolution 4 cm^{-1}). Other details of spectra acquisition were reported earlier [28,30].

2.3. Analyses of bacterial samples for metal cations

Metal cations (Co, Cu and Zn) were determined in the same bacterial samples that were used for spectroscopic measurements. Precisely weighed portions of the dried bacterial biomass (10–37 mg) were digested as described earlier [29,31] and analysed using a Hewlett-Packard ICP-MS spectrometer (model 4500). Unless indicated otherwise, all measurements were performed at ambient temperature (295 ± 3 K).

2.4. Sample preparation and EMS measurements

For EMS measurements, the culture of *A. brasilense* Sp245 was grown as described above in the standard phosphate-malate mineral medium supplemented with 5 mM NH_4Cl as a nitrogen source (pH 6.9). The cell density in the growing culture was controlled by spectroturbidimetric measurements [31] up to ca. 2.4×10^8 cells ml^{-1} (approximately mid-exponential growth phase). Optical microscopic observations confirmed the motility of all cells in the culture. The culture obtained was stored for 1 day in Eppendorf tubes at 4 °C and, just prior to adding $^{57}Co^{2+}$, incubated at room temperature (20–23 °C) for 1 h. To prepare dead cells, aliquots of the culture were kept in small plastic Eppendorf tubes (ca. 1.5 ml) in a water bath at 90 °C for 1 h and then, just prior to adding $^{57}Co^{2+}$, cooled down to room temperature. Aliquots of the cell suspensions (1.0 ml), grown and treated as above, were then placed into PTFE sample holders each containing 1 mCi of radioactive $^{57}CoCl_2$ free from natural Co^{2+} (obtained from the Centre for Radionuclide Diagnostics, Moscow State University, Moscow, Russia), that had been dried from aqueous solution (final $^{57}Co^{2+}$ concentration ca. 2×10^{-6} M), thoroughly mixed, closed to prevent evaporation, and after 2 or 60 min of incubation at room temperature the corresponding samples were rapidly frozen in liquid nitrogen at ca. 80 K (further used for EMS measurements either as a frozen suspension or as a freeze-dried powder). A sample with dead cells prepared as above was processed identically (60 min of incubation with $^{57}Co^{2+}$; rapidly frozen suspension). A similar sample (60 min of incubation with $^{57}Co^{2+}$) was prepared using transparent cell-free supernatant liquid separated from the bacterial cells by centrifugation (3000 rpm, rotor radius 50 cm, 50 min) immediately after growth and rapidly frozen in liquid nitrogen.

EMS measurements were performed by placing the ^{57}Co-containing sample (source) in a cryostat filled with liquid nitrogen (at ca. 80 K) using a conventional constant-acceleration Mössbauer spectrometer (absorber $K_4[Fe(CN)_6]\cdot 3H_2O$) combined with a PC-operated multichannel analyser. Standard PC-based statistical analysis consisted of fitting the experimental data obtained (converted into a form compatible with that of absorption ^{57}Fe Mössbauer measurements) as a sum of Lorentzians using a least squares minimisation procedure, which resulted in χ^2 minimisation (in all cases, $1 < \chi^2 \leq 1.2$). This enabled determination of the isomer shift (IS; relative to α-Fe at room temperature), quadrupole splitting (QS), linewidth (i.e. full width at half maximum, FWHM) and relative areas of spectral components (S_r). Other details of methodology and data treatment were reported elsewhere [16,24,25].