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# Mössbauer spectroscopic study of $^{57}$Fe metabolic transformations in the rhizobacterium *Azospirillum brasilense* Sp245

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**Abstract** Preliminary $^{57}$Fe transmission Mössbauer spectroscopic data were obtained for the first time for live cells of the plant-growth-promoting rhizobacterium *Azospirillum brasilense* (wild-type strain Sp245) grown aerobically with $^{57}Fe^{III}$–nitrilotriacetate (NTA) complex as a sole source of iron. The results obtained have shown that live cells actively reduce part of the assimilated iron(III) to iron(II), the latter amounting up to 33 % of total cellular iron after 18 h of growth, and 48 % after additional 3 days of storage of the dense wet cell suspension in nutrient-free saline solution in air at room temperature (measured at 80 K). The cellular iron(II) was found to be represented by two quadrupole doublets of different high-spin forms, while the parameters of the cellular iron(III) were close to those typical for bacterioferritins.

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## 1 Introduction

Rhizobacteria of the genus *Azospirillum*, and the species *A. brasilense* in particular, have been attracting the growing attention of researchers for the last decades owing to a number of their beneficial traits [1–3]. These bacteria can form rhizospheric associative symbioses with various higher plants, promoting their growth and development via several mechanisms including (but not limited to) phytohormone production and atmospheric nitrogen fixation [2, 3]. Our recent studies on metabolic responses of *A. brasilense* strains (with different ecological behaviour) to various environmental factors involved a range of spectroscopic techniques (see, e.g. [4–8]), including $^{57}$Co emission Mössbauer spectroscopy [6–8].

The information on the uptake and metabolism of iron in azospirilla available in the literature is scarce and evidently insufficient [9–12]. In this work, $^{57}$Fe transmission Mössbauer spectroscopy was used (for the first time for bacteria of the genus *Azospirillum*) to monitor metabolic transformations of iron species assimilated from the culture medium in live cells of the bacterium *A. brasilense* (wild-type strain Sp245).

## 2 Experimental

The bacterium *A. brasilense* Sp245 (from the Collection of IBPPM RAS, Saratov) was cultivated at 31 °C for 18 h under aeration on a rotary shaker (180 rpm) in a standard phosphate–malate medium [4–8] with 0.5 g·l$^{-1}$ $NH_4Cl$ as a source of bound nitrogen and 0.070 mM $^{57}Fe^{III}$–NTA complex (4.0 mg·l$^{-1}$ $^{57}Fe^{III}$) as a sole source of iron. The cells were then separated from the culture medium by centrifugation (2370·*g*, 30 min) and washed 3 times with sterile saline solution (aqueous 0.85 % NaCl). Part of the resulting wet suspension was rapidly frozen in liquid nitrogen for Mössbauer measurements. The other part (closed to prevent drying) was stored in the sterilised sample holder of the Mössbauer spectrometer at room temperature for 3 days (no Mössbauer spectrum was obtained from this sample at room temperature within 3 days) and then rapidly frozen in liquid nitrogen for further Mössbauer measurements at 80 K.

Mössbauer spectra were obtained using a conventional Mössbauer spectrometer WISSEL (MDU-1200 drive with MVT 1000 velocity transducer) for the resulting frozen aqueous suspensions. Samples were kept in a specially designed "cold finger" cryostat (with a window for $\gamma$-rays) filled with liquid nitrogen (at $T = 80$ K) as reported elsewhere [13]. The experimental data obtained using a conventional scintillation detector and a computer-operated multichannel analyser (MSPCA Analyser Mössbauer Spectrometer Control and Acquisition Unit) as described elsewhere [7, 8, 13] were processed using the MOSSWINN program [14]. Standard computer-based statistical analysis included fitting the experimental data to a sum of Lorentzians using a least squares minimisation procedure for $\chi^2$. The values of isomer shift ($\delta$; relative to $\alpha$-Fe at room temperature), quadrupole splitting ($\Delta$),

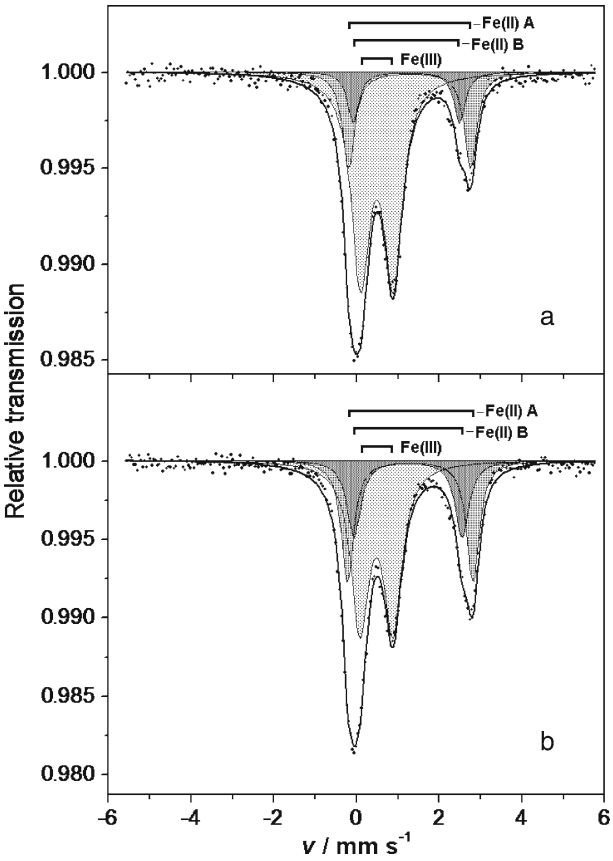


**Fig. 1** Mössbauer spectra (measured at $T = 80$ K) of *A. brasilense* Sp245 live cells grown with $^{57}Fe^{III}$–NTA complex as a sole source of iron, centrifuged and rapidly frozen in liquid nitrogen **a** after 18 h of growth (Sample 1) or **b** after additional 3 days of storage (wet nutrient-free suspension) at room temperature (Sample 2; see also Table 1). The positions of the corresponding quadrupole doublets are shown by square brackets above the spectra

linewidth (W; full width at half maximum) and relative areas of spectral components ($A$) were thus calculated for each spectrum.

## 3 Results and discussion

Mössbauer spectra (measured at $T = 80$ K) obtained for live cells of *A. brasilense* Sp245 rapidly frozen after 18 h of growth in the culture medium (Sample 1) or after additional three days of storage of the dense wet nutrient-free cell suspension at room temperature (Sample 2) are presented in Fig. 1. Mössbauer parameters calculated from the spectroscopic data are listed in Table 1. Each of the two spectra is featured by three quadrupole doublets; two of them, with larger quadrupole splitting values ($\Delta \sim 3.0$ and 2.6 mm/s) and relevant isomer shift values ($\delta \sim 1.3$ and 1.2 mm/s, respectively), evidently correspond to two high-spin hexacoordinated iron(II) forms (doublets A and B, respectively; see Fig. 1), representing different types of coordination microenvironments (i.e., different complexes) of $^{57}Fe^{2+}$ with O- and/or N-coordinated donor atoms, similar to those found for *Escherichia coli*, another Gram-negative bacterium [15].

**Table 1** Mössbauer parameters[a] (measured at $T = 80$ K) calculated for *A. brasilense* Sp245 live cells grown with $^{57}Fe^{III}$–NTA complex as a sole source of iron, centrifuged and rapidly frozen in liquid nitrogen after 18 h of growth (Sample 1) or after additional 3 days of storage (wet nutrient-free suspension) at room temperature (Sample 2; see also Fig. 1)

| Mössbauer parameters for Fe components | Sample 1 frozen after 18 h of growth | Sample 2 frozen after additional 3 days at room temperature |
|---|---|---|
| $Fe^{2+}$(A) | | |
| *A*, % | **22(3) %** | **28(3) %** |
| $\delta$, mm/s | 1.28 (1) | 1.30 (1) |
| $\Delta$, mm/s | 2.95 (3) | 3.03 (2) |
| W (FWHM), mm/s | 0.37 (2) | 0.37 (2) |
| $Fe^{2+}$(B) | | |
| *A*, % | **11(3) %** | **20(3) %** |
| $\delta$, mm/s | 1.20 (2) | 1.24 (1) |
| $\Delta$, mm/s | 2.57 (4) | 2.61 (3) |
| W (FWHM), mm/s | 0.34 (4) | 0.41 (3) |
| $Fe^{3+}$(C) | | |
| *A*, % | **67(1) %** | **52(1) %** |
| $\delta$, mm/s | 0.49 (1) | 0.48 (4) |
| $\Delta$, mm/s | 0.79 (6) | 0.80 (1) |
| W (FWHM), mm/s | 0.54 (1) | 0.52 (1) |

[a]Errors (in the last digits) are given in parentheses: $\delta$, isomer shift (relative to $\alpha$-Fe at ambient temperature); $\Delta$, quadrupole splitting; W, full line width at half maximum; *A*, partial resonant absorption areas of spectral components which represent relative contents of the corresponding Fe forms assuming a common recoilless fraction for all forms

In a fresh wet cell culture (grown aerobically on a shaker for 18 h, separated by centrifugation after washing with sterile saline solution and then rapidly frozen), iron(II) reached 33 % of the total cellular Fe. After storage of the wet dense biomass (separated from nutrients and culture components by washing with saline solution and centrifugation) at room temperature (rapidly frozen after additional three days), relative iron(II) content, featured by virtually the same two forms, further increased up to 48 %. It would be of interest to elucidate in further studies what could be the reason for this microaerophilic bacterium [1, 2], which was evidently under a severe 'culture density stress' for a few days under nutrient-free aerobic conditions, to continue intracellular reduction of iron(III), assimilated from $^{57}Fe^{III}$–NTA complex in the culture medium, to iron(II).

The ferric component in each of the samples was represented by a quadrupole doublet ($\delta \sim 0.5$ mm/s; $\Delta \sim 0.8$ mm/s) with a slightly increased line width (over 0.5 mm/s). According to the Mössbauer parameters, it could be attributed to bacterial ferritin-like components similar to those observed for other microorganisms [15–17].

## 4 Conclusions

Live cells of *A. brasilense* Sp245 have been found to actively reduce iron(III) in the course of its assimilation from the medium where it was present as $^{57}Fe^{III}$–NTA complex. After 18 h of growth, the reduced iron(II) reached 33 % of the total cellular iron, while after additional three days of storage of the nutrient-free wet dense cell suspension, the fraction of iron(II) reached 48 %. In both cases,

iron(II) was represented by two high-spin hexacoordinated forms, both of which tended to increase upon storage. The ferric component in each of the samples was represented by a quadrupole doublet with a slightly increased line width, which could be attributed to bacterial ferritin-like components. This is the first report on Mössbauer spectroscopic monitoring of $^{57}$Fe species in cells of the bacteria of the genus *Azospirillum*.

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