

Improved *In Situ* Tracking of Rhizosphere Bacteria Using Dual Staining with Fluorescence-Labeled Antibodies and rRNA-Targeted Oligonucleotides

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ABSTRACT

Fluorescence-labeled antibodies and oligonucleotides were used simultaneously for the *in situ* identification of bacteria in mixed cultures, as well as in the rhizosphere of inoculated plants. Counterstaining was performed with 4'-6-diamidino-2-phenylindole (DAPI), and scanning confocal laser microscopy or epifluorescence microscopy with a charge-coupled device (CCD) camera were used for detection of individual cells. This strategy gave insight into the relative abundance of an inoculated strain, enabled the exact localization of single cells, and allowed the estimation of the metabolic activity of the bacteria in a complex specimen. Using a strain-specific monoclonal antibody for *Azospirillum brasilense* Wa3, we could identify this particular strain in root samples of inoculated wheat plantlets. Strain Wa3, as well as other bacteria colonizing the rhizosphere, were stained simultaneously with rRNA-targeted, fluorescence-labeled oligonucleotide probes. In a co-inoculation experiment with *A. brasilense* strains Sp7 and Wa3, it was demonstrated by *in situ* identification and quantitative chemoluminescence ELISA that strain Sp7 outcompeted strain Wa3. The combined application of fluorescently labeled antibodies and oligonucleotides should be generally applicable for monitoring specific bacterial strains, within the background of the same species, in relation to the total microbiota.

Introduction

Since the first report on staining of single microbial cells with fluorescence-labeled, rRNA-directed oligonucleotides

by DeLong et al. [11], the use of this technique has rapidly increased in microbial ecology. The major advantages of this method include the detection of specifically stained single cells in complex media [2], their phylogenetic affiliation [25] and an estimation of their *in situ* physiological activity [20]. The use of these probes to detect target organism(s) is even possible if the organism has not been previously cultured

Table 1. List of the bacterial strains used in this study, their origin, and the abilities of the antibodies and oligonucleotide probes applied for specific staining

Strain	Origin	Staining with:						
		Antibodies			rRNA probes			
		3.1	Bo 2-33	pAs Hsp	Eub338	ALF1b	BET42a	AB
<i>A. brasilense</i> Sp7	<i>Digitaria</i> roots [42]	+	–	–	+	+	–	+
<i>A. brasilense</i> Sp245	Wheat roots [6]	–	–	–	+	+	–	+
<i>A. brasilense</i> Wa3	Wheat roots [9]	–	+	–	+	+	–	+
<i>A. lipoferum</i> Br17	Maize roots [42]	–	–	–	+	+	–	–
<i>H. seropedicae</i> Z67	Rice roots [5]	–	–	+	+	–	+	–
<i>H. rubrisubalbicans</i> M4	Sugar cane leaves [7]	–	–	–	+	–	+	–

[3]. Fluorescently labeled oligonucleotide probes have been used to monitor microbial communities e.g. in aquatic eco-systems [24], activated sludge [47], biofilms [29], sediments [39], and rhizosphere soil [4], as well as endosymbionts in protists [13], bivalves [12], and root nodule homogenates [17].

However, due to the relatively high degree of conservation within the rRNAs, the occurrence of signature sequences at the strain level is limited. Probes are usually designed to detect organisms at the species level or higher taxonomic units. Antibiotic-resistant mutants [10] or genetically engineered derivatives carrying reporter genes (for review see refs. 30, 41) have typically been used to monitor the fate of specific introduced strains. However, antibiotic resistance–based strategies depend on a cultivation step, cannot be used for in situ studies, and can be affected by a high natural background of the phenotype used for selection [44]. Tracking genetically modified strains can be biased by gene or plasmid transfer to the indigenous microbiota [43] and the ecological fitness of these strains may not be comparable to the parental wild type [45]. Furthermore, the use of such genetically engineered derivatives for field experiments is limited because of biosafety considerations.

Antibodies labeled with a fluorescent dye have served as a tool for the specific in situ staining of bacterial cells for more than 30 years [37]. If monoclonal antibodies (mAbs) or polyclonal antisera (pAs) suitable for a reliable in situ identification are used [36], even strain-specific tracking is possible and the sensitivity of immunofluorescence detection of microorganisms can be compared to that of PCR [46,23]. Schank et al. [31] used a pAs to investigate the colonization of grass roots by *Azospirillum brasilense* Cd. Recently, the localization of an introduced *A. brasilense* Sp7 in the rhizosphere of wheat was determined using a strain-specific mAb

and scanning confocal laser microscopy [33]. *Azospirillum* species are common bacteria in the rhizosphere of cereals, mainly in tropical countries, and are considered as potential inoculants in agriculture [26]. Because they exhibit strain-specific colonization behavior in the rhizosphere [27], it is necessary to obtain information about the fate of an introduced strain before selecting those strains that are most suited for application. The vast majority of field inoculation experiments with *Azospirillum* strains have been conducted in regions where this organism occurs naturally in the soil. Therefore, the interaction between different strains within one species, as well as between the introduced strain and the total rhizosphere microbiota, is of great importance. The simultaneous use of antibodies and rRNA-directed probes for investigating these relationships, has a number of potential advantages. The antibody would specifically detect the strain to be monitored, whereas an oligonucleotide probe could be used to cover the whole species or higher taxonomic unit. It would also allow an estimation of the in situ physiological status of the target bacteria. However, neither protocols for such a dual staining nor their application has been reported. We describe herein methods for multiple staining and their application in studies of rhizosphere colonization by *Azospirillum brasilense*.

Materials and Methods

Bacterial Strains and Media

The bacterial strains used in this study are listed in Table 1. Strains were grown in Luria broth (LB) at 33°C. Bacteria used in inoculation experiments were grown at 33°C in a nitrogen-free minimal medium containing succinate as sole carbon and energy source [4].

Fig. 1 (opposite). CCD-recorded images of mixed bacterial laboratory cultures after triple staining with antibodies, rRNA-directed oligonucleotides, and DAPI. Bars equal 12 μm . (a) Mixture of *Herbaspirillum seropedicae* Z67, *H. rubrisubalbicans* M4, and *Azospirillum brasilense* Sp7; staining: fluorescein-labeled pAs specific for *H. seropedicae* (green fluorescence), TRITC-labeled probe BET42a (red fluorescence), and DAPI (blue fluorescence). *Azospirillum brasilense* Sp7 (egg-shaped) is only stained blue by DAPI. *H. rubrisubalbicans* appears purple due to the overlay of blue and red fluorescence from DAPI and rRNA-probe. *H. seropedicae* additionally has a green mantle due to the antibody which results in white

when combined with rRNA-probe and DAPI. (b) Mixture of *Azospirillum brasilense* strains Wa3 and Sp245, and *A. lipoferum* Br17; staining: Texas Red-labeled mAb Bo2-33 specific for strain Wa3 (red fluorescence), FLUOS-labeled probe AB specific for the species *Azospirillum brasilense* (green fluorescence), and DAPI (blue fluorescence). The turquoise color of the *A. brasilense* strains results from overlay of DAPI- and probe AB-conferred fluorescence; strain Wa3 is mantled with red color representing the surface-bound antibody and appears white in the center due to the overlay of red and turquoise.

Oligonucleotide Probes and Antibodies

The following oligonucleotide probes were used: (1) EUB338, complementary to a sequence stretch of the 16S rRNA specific for the domain Bacteria [1]; (2) ALF1b, complementary to a region in the 16S rRNA conserved in the alpha subclass of Proteobacteria and a few other bacteria [25]; (3) BET42a, complementary to a region in the 23S rRNA of beta subclass Proteobacteria [25]; (4) AB, complementary to a species-specific sequence of the 23S rRNA of *Azospirillum brasilense* [21]. Oligonucleotide probes were synthesized with a C6-TFA aminolinker [6-(trifluoroacetyl-amino)-hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite] at the 5' end (MWG Biotech, Ebersberg, Germany). They were labeled with tetramethylrhodamine-5-isothiocyanate (TRITC) (Molecular Probes, Eugene, Ore.) or 5(6)-carboxyfluorescein-N-hydroxysuccinimide ester (FLUOS) (Boehringer Mannheim, Penzberg, Germany), purified as described by Amann et al. [2] and stored at -20°C .

For immunological staining of the bacterial cells, strain-specific mAbs for *A. brasilense* strains Sp7 and Wa3 and a species-specific pAs for *Herbaspirillum seropedicae* were used. mAb-producing hybridoma cell lines were obtained by fusion of the myeloma cell line X63-Ag8.653 with B-lymphocytes of 3- to 6-month-old rats, which had been stimulated 1–5 times by immunization with 2×10^8 living cells [15]. mAbs with a high specificity for the respective strain were purified by hydroxyapatite column chromatography [40] and stored at -20°C . The experiments described here were carried out with Bo 2-33, which is a strain-specific mAb of the IgG₃ subclass for *A. brasilense* Wa3. The antigenic determinant of this mAb is located in the LPS layer (M. Schlöter, unpublished result). mAb 3.1, which is specific for *A. brasilense* Sp7 [35], was also used for quantification of reextracted bacteria from the rhizosphere by a chemoluminescence-based immunoassay. The strain specificity of the mAbs was checked by cross reaction experiments using the ELISA technique with *Azospirillum brasilense* strains, with representative strains of other *Azospirillum* species, and with a broad range of Gram-negative and Gram-positive soil bacteria. Further characterization of antigenic epitopes of the mAbs has been done by protein and LPS analysis combined with western blotting experiments as described in ref. 36.

The pAs was obtained from a 12-month-old female New Zealand rabbit which had been immunized 5 times with 10^9 living cells

of *H. seropedicae* Z67 (ATCC 35892) mixed with an equal volume of Freund's adjuvant [8]. The pAs was purified with a protein A column [14] and a preincubation technique using an immunoaffinity column with whole cells of *Pseudomonas fluorescens* as antigenic determinant [18]. The pAs showed cross reaction with 7 tested *H. seropedicae* strains but not with 4 *H. rubrisubalbicans* strains and 12 strains belonging to the rhizosphere relevant genera *Azospirillum*, *Rhizobium*, *Pseudomonas*, and *Bacillus*. The antigenic determinants are located on the cell surface and include protein and LPS structures (M. Schlöter, unpublished results).

The specificities of all oligonucleotide probes and antibodies used to stain the bacterial strains are summarized in Table 1.

The DNA-specific dye 4',6-diamidino-2-phenylindole (Sigma, Deisenhofen, Germany) was stored as an aqueous stock solution 0.35 mg ml^{-1} at 4°C .

Inoculation and Growth of Wheat Seedlings and Preparation of Samples and Slides

Seeds of the wheat cultivar PF839197 were germinated for 2 days on moistened paper at room temperature. Afterward, the seeds were transferred to 50-ml tubes filled with approximately 40 g of unsterilized soil (silty loam). The tubes were drained in order to avoid waterlogged conditions. At the time of planting, inoculation was performed: *A. brasilense* cells were harvested at late-logarithmic growth phase, washed in sterile phosphate buffered saline (PBS, pH 7.2), and approximately 10^8 cells were added to each tube. The seedlings were grown in a greenhouse at 28°C and soil was moistened every 3 days with 1.5 ml of distilled water.

Wheat seedlings were also grown in agar tubes containing nitrogen-free minimal medium, as described above, except that succinate was omitted and 8 g agar per liter was added. The medium was autoclaved in the tubes and held at 42°C . Approximately 10^8 washed cells from a culture of the desired strain grown until late-logarithmic phase were added. The tubes were gently shaken and put on ice. After the agar had solidified, one sterilized germinated seed was placed on the surface of each tube with sterile forceps, and the tube was sealed with an autoclaved cotton plug. Seeds were surface sterilized using sodium hypochlorite and hydrogen peroxide, as described previously [4].

Figure 1. (legend see previous page)

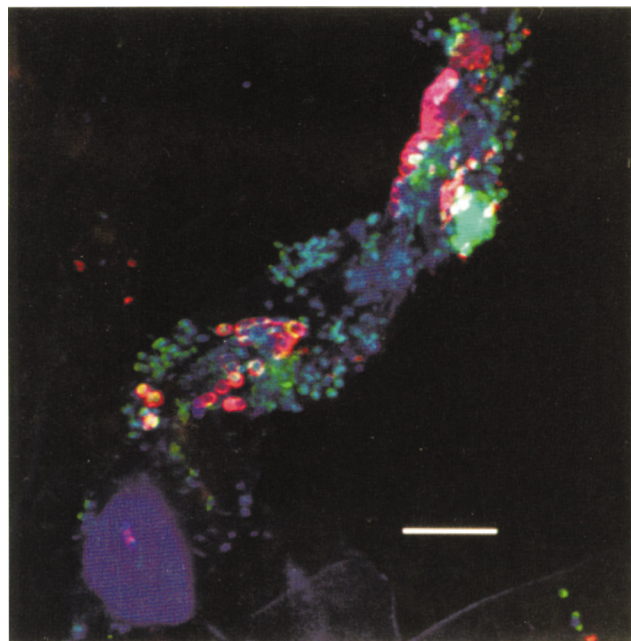
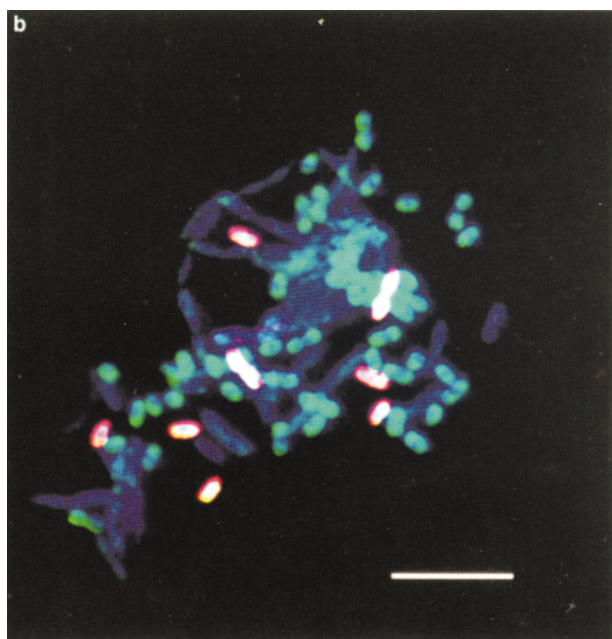
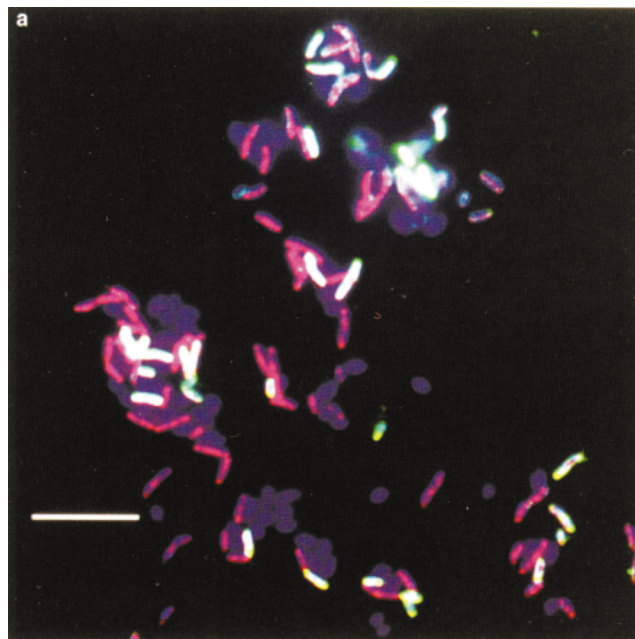


Fig. 2. Tightly colonized root hair from a wheat seedling grown in unsterilized soil and inoculated with *A. brasilense* Wa3; staining: Texas Red-labeled mAb specific for *A. brasilense* Wa3 (red fluorescence), FLUOS-labeled bacterial consensus probe Eub338 (green fluorescence), and DAPI (blue fluorescence). The large blue object at the lower left is the nucleus of the root hair cell. Colored projection reconstructed from three monochrome z-sequences recorded with SCLM at excitation wavelengths of 364, 488, and 543 nm, respectively. Depth 10 μ m. Bar equals 10 μ m.

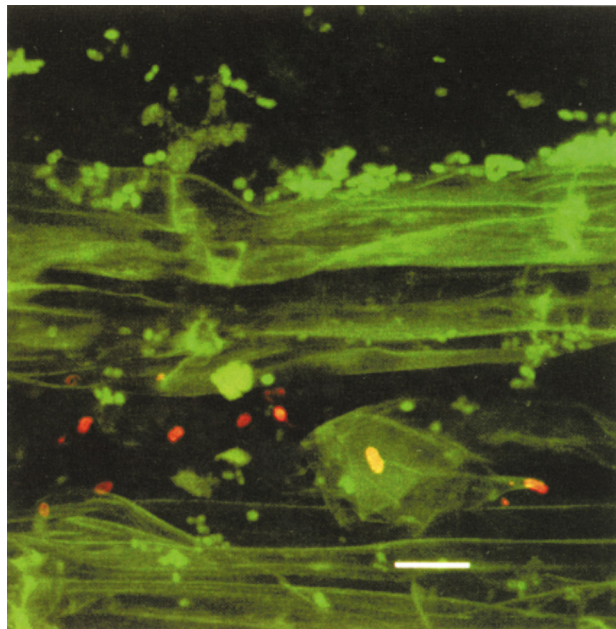


Fig. 3. Root surface of an agar-grown wheat plantlet co-inoculated with *A. brasilense* strains Sp7 and Wa3; staining: Texas Red-labeled mAb specific for *A. brasilense* Wa3 (red fluorescence) and FLUOS-labeled probe ALF1b (green fluorescence) covering both strains. Reconstructed projection of two monochrome z-sequences recorded with SCLM at excitation wavelengths of 488 and 543 nm, respectively. Depth 14 μ m. Bar equals 10 μ m.

Fixation of laboratory cultures was done with 3% paraformaldehyde at 4°C overnight, following the protocol of Amann et al. [2]; Root samples were harvested between 9 and 14 days after inoculation. The root pieces were fixed with paraformaldehyde and dehydrated at room temperature before mounting them on slides [4].

Protocol for In Situ Staining with Indirect Immunofluorescence and Hybridization with Oligonucleotide Probes

To reduce nonspecific binding of the antibodies to the root surface, the slides with the fixed root samples were blocked for 30 min at 37°C with 40 µl of PBS containing 3% bovine serum albumin. After removing the blocking reagent, the samples were immersed in an appropriate dilution of the mAb or pAs for 30 min at 37°C. The appropriate dilution was determined in ELISA experiments by step-wise dilution of the mAb or pAs solution. The dilution with the highest ratio between specific signal and nonspecific background was chosen for further experiments. After three washing steps (5 min each) on a shaker with washing solution (1/10 PBS and 0.1% fetal calf serum [Biochrom, Berlin, Germany]; also used for dilution of antibodies), 40 µl of a 1:300 dilution of the biotinylated second antibody (Amersham, Braunschweig, Germany) were added for 45 min at 37°C. After three washing steps, the samples were incubated with 40 µl of a 1:50 dilution of streptavidin conjugate (streptavidin-fluorescein or streptavidin-Texas Red, Amersham, Braunschweig, Germany) for 30 min at 37°C. After five washing steps, the slides were immersed for 5 min in PBS containing 0.2% (v/v) Tween 80. After rinsing with distilled water, the slides were fixed for 20 min in a chilled mixture of methanol and acetone (1:1) and then allowed to air dry, before the in situ hybridization followed.

Hybridization was performed as published by Manz et al. [25]. The hybridization buffer (0.9 M NaCl, 20 mM Tris hydrochloride [pH 7.2], 0.01% SDS, 5 mM EDTA) contained 10% formamide when probe AB was used, 20% formamide for probe ALF1b, and 35% formamide for probe BET42a to guarantee stringent conditions. Two microliters of probe working solution were added. The slides were incubated for 2 h at 46°C in an equilibrated humidity chamber. Unbound probe molecules were removed with 5 ml of washing solution (20 mM Tris hydrochloride [pH 7.2], 0.01% SDS, 5 mM EDTA, various amounts of NaCl), and the slides were immersed in 50 ml of washing solution at 48°C for 20 min. NaCl added to the washing solution was zero for probe BET42a, 0.18 M for probe ALF1b, 0.5 M for probe AB, and 0.9 M for probe Eub338. The slides were then rinsed with distilled water and air dried. If counterstaining with DAPI was desired [28], the stock solution was diluted 500-fold in PBS, and 20 µl of the working solution was applied to each root piece. The slides were incubated for 5 min at 28°C, rinsed with distilled water, air dried and mounted in anti-fading solution [19].

Staining experiments using mixed bacterial samples were performed on gelatine-coated slides on which a mixture of fixed bacteria was spotted [2]. The staining procedure was performed as described above.

Imaging of Stained Samples

An LSM 410 inverted scanning confocal laser microscope (Zeiss, Oberkochen, Germany) equipped with three lasers (Ar-ion, UV; Ar-ion, visible; He-Ne) supplying excitation wavelengths at 365, 488, and 543 nm, respectively, was used to record optical sections. A 100× oil immersion lense (NA 1.3) was used. Monochrome sequences of images were taken along the optical axis (z-axis) with increments between 0.7 and 1.2 µm. Sequentially recorded monochrome images or projections of monochrome z-sequences were assigned to the respective fluorescence color and then merged to a true color red/green/blue (rgb) display. All image combining and processing was performed with the standard software provided by Zeiss. For a review of the principles of scanning laser microscopy, see Shotton and White [38].

The microscope was also connected to a Photometrics AT 200 cooled charge-coupled device camera equipped with a Kodak KF 1400 sensor (Photometrics, Tucson, Ariz.) to record high-resolution epifluorescence images. For epifluorescence microscopy, a high-pressure mercury bulb was used. Filters used were a band-pass 395–440 nm for blue fluorescence, a bandpass 510–525 nm for green, and a longpass 564 nm for red fluorescence (Chroma, Brattleboro, VT.).

Quantification of Bacteria Extracted from the Rhizosphere with Chemoluminescence ELISA

Roots from agar-grown wheat plantlets were harvested as described above and representative samples of root tips, root hair zone, old parts close to the root base, and secondary roots were prepared for in situ investigation. The remaining parts were weighed and the bacteria were detached from the root matrix by grinding [34]. A quantitative, chemoluminescence-based immunoassay was performed in PE microtiter plates (Merlin, Hamburg, Germany) using a secondary antibody coupled with peroxidase and luminol (Amersham, Braunschweig, Germany) according to Schlöter et al. [32].

Results and Discussion

Combination of Immunofluorescence Staining and Whole Cell Hybridization with Oligonucleotide Probes

The staining protocol presented here enabled the concomitant and specific detection of bacteria with both oligonucleotide probes and antibodies with a good fluorescence signal intensity. Figure 1a shows a mixture of *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, and *Azospirillum brasilense* Sp7 after staining with a polyclonal antiserum (pAs) specific for *H. seropedicae*, probe BET42a specific for the beta subclass of Proteobacteria, and DAPI. Whereas the antiserum detects only *H. seropedicae*, the probe hybridized to both *Herbaspirillum* species that belong to the beta subclass of Proteobacteria. The image clearly shows the difference between rRNA

probe- and surface-directed antibody staining; whereas the probe signal comes from the cell lumen, the antibody-conferred fluorescence surrounds the cells. *A. brasilense* was stained exclusively by DAPI. If the bacterial consensus probe Eub338 was applied to the same mixture of organisms, all three species could be detected (not shown). In another experiment (Fig. 1b), *A. brasilense* strain Wa3 was specifically stained by the monoclonal antibody (mAb) Bo 2-33 in the presence of strain Sp245 of the same species, and both strains could be distinguished from *A. lipoferum* by hybridization with the species-specific probe AB.

The binding of antibodies to the respective surface antigens was obviously neither affected by the temperatures used during the subsequent hybridization and washing procedures nor by the detergent, salt, or formamide concentrations used to guarantee stringent hybridization conditions. A second fixation step, after immunolabeling of the specimen, enabled unhindered access of the oligonucleotides to the interior of the target cells without interference with the immunolabeling. If this step was omitted, no probe hybridization signal could be detected. A mixture of methanol and acetone was superior to 4% paraformaldehyde for postfixation, i.e., the latter resulted in incomplete and weaker staining with the rRNA-probes. If the hybridization with oligonucleotides was performed before immunolabeling, the fluorescence intensity conferred by the oligonucleotide probe was very weak, if any signal was obtained at all. Probably in this case, a large amount of bound oligonucleotide had dissociated and been washed away due to the low salt conditions during the antibody staining procedure.

In general, the signal intensity conferred by antibody staining was much higher than the signals resulting from in situ hybridization with oligonucleotide probes. This was also observed if the specific staining was performed with probes or antibodies separately, and, therefore, was not due to the combination of the methods. Therefore, when colored images were rearranged from the original greyscale files, the signal intensity resulting from bound fluorescence-labeled antibodies had to be dimmed in relation to the fluorescence conferred by a probe or DAPI. This was done using the image processing routines of the Zeiss LSM software.

Detection and Identification of Inoculated Bacteria in the Rhizosphere Using Antibodies and rRNA-Probes

To demonstrate the capability of this multiple staining protocol for in situ detection, we applied the same procedure to root samples from inoculated wheat seedlings. Figure 2

shows a root hair from a plantlet grown in nonsterile soil, whose surface is colonized by a mixed bacterial community including *A. brasilense* Wa3 which had been inoculated in this case and was detected by the strain-specific monoclonal antibody. The sample was hybridized with the bacterial consensus probe Eub338. Approximately one-third of the bacterial community exhibited a probe signal, and an even greater proportion in the specific case of the imaged root hair. The proportion of Wa3 cells that showed probe-conferred fluorescence was in the same range. Theoretically, the remaining Wa3 cells could indicate limited penetration of the oligonucleotide through antibody-covered cell walls. However, sufficient access of probes to the target site was demonstrated in the laboratory experiments with pure cultures (see above). More likely, some of the bacterial cells had a ribosome content too low for a detectable probe signal. The direct relationship between probe-conferred signal and cellular rRNA content has been previously reported [11, 22]. Because the rRNA content of a cell is a general measure of metabolic activity [20, 47], the proportion of *Azospirillum* cells not detectable by the oligonucleotide probe were probably inactive. Whether the cells exclusively stained by DAPI were quiescent bacteria, had a cell wall not penetrable by the rRNA probe, or lacked the target sequence cannot be concluded. From experiments in bulk soil it is known that less than 1% of the bacteria have detectable metabolic activity [16]. In the rhizosphere this proportion was found to be larger [4], probably due to organic substrates supplied by root exudates.

Monitoring of Co-Inoculated A. brasilense Strains

Axenic wheat seedlings were grown in mineral medium and co-inoculated with *A. brasilense* strains Sp7 and Wa3. After harvesting and preparing the roots, both strains could be distinguished on the investigated samples. Whereas only the latter strain was detected by the strain-specific mAb Bo2-33, both strains were detected by the fluorescently labeled rRNA-probe, ALF1b, specific for alpha *Proteobacteria*. On root samples from seedlings inoculated only with strain Sp7, no antibody staining occurred. When only strain Wa3 had been inoculated, all detected bacteria exhibited antibody-conferred fluorescence. In uninoculated sterile control plants, no bacteria were detected either by the rRNA-probe, the antibody, or DAPI. In monoxenic plants, strains Sp7 or Wa3 formed tight clumps and layers on the root surface and on root hairs (not shown). The root tips were free from bacteria. When both strains had been coinoculated, only Sp7

exhibited abundant growth on the root surface. It exceeded the number of Wa3 cells by several-fold (Fig. 3). In co-inoculated plants, Wa3 occurred mainly as small groups or single cells. When inoculated separately, only a small proportion of the cells of Sp7 or Wa3 lacked rRNA probe-conferred fluorescence. However, after co-inoculation, the relative proportion of Wa3 cells not stained by the rRNA probe was significantly larger. This was not the case with Sp7 cells. These observations may indicate a decreased metabolic activity of Wa3 after co-inoculation at the time of harvesting.

The findings of the in situ observations were confirmed by a quantitative chemoluminescence ELISA using two strain-specific monoclonal antibodies (Table 2). The overall population density of *A. brasilense* on roots of plants inoculated with either strain Sp7 or Wa3 or both was in the same range of several times 10⁶ cfu g⁻¹ wet root. However, when co-inoculated with Sp7, strain Wa3 accounted for only 10% of the detected *A. brasilense* cells. Obviously, strain Wa3 was outcompeted by strain Sp7. These findings are of particular interest because the type strain Sp7 was originally isolated from *Digitaria* roots [42], whereas strain Wa3 is a wheat rhizosphere isolate [9]. Thus, it cannot be concluded from the original plant source of a strain whether it will be an effective rhizosphere colonizer of this plant species under competitive conditions.

Conclusions

The combination of immunofluorescence and whole cell-hybridization with fluorescently labeled phylogenetic oligonucleotide probes expands the capability of in situ staining assays. A simultaneous detection of bacteria at different specificity levels ranging from strain to domain level is now

Table 2. Quantification of *A. brasilense* strains Sp7 and Wa3 from roots of 10-day-old wheat plantlets by chemoluminescence ELISA

Inoculum	Root colonization (cfu/g wet root) ^a	
	<i>A. brasilense</i> Sp7	<i>A. brasilense</i> Wa3
<i>A. brasilense</i> Sp7	2 × 10 ⁶	n.d. ^b
<i>A. brasilense</i> Wa3	n.d.	4 × 10 ⁶
Coinoculation	2 × 10 ⁶	3 × 10 ⁵
Uninoculated control	— ^c	—

^a Detection of the bacteria was performed with the strain-specific mAb Bo2-33 for strain Wa3, and mAb 3.1 for strain Sp7, respectively. The standard deviation of the assay was less than 5%.

^b n.d., not determined.

^c —, not detectable.

possible, and should enable the monitoring of a strain or species of interest within natural communities that include closely related organisms. This is of crucial importance in inoculation experiments with plant growth-promoting bacteria, where the colonization efficiency has to be monitored in the presence of, and in relation to, indigenous strains of the same species. In future experiments, structure-function relationships within the natural microbial community may be elucidated by using nucleotide probes targeted against structural genes and antibodies against surface-exposed or cytoplasmatic enzymes. The new imaging techniques, such as confocal microscopy and image processing, successfully complement these probing tools.

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