Prokaryotes (2006) 5:141–150
DOI: 10.1007/0-387-30745-1_7

CHAPTER 3.1.6

The Genus *Herbaspirillum*

MICHAEL SCHMID, JOSE IVO BALDANI AND ANTON HARTMANN

Historical Aspects

Owing to its cell form, growth behavior and habitat within grass roots, the first isolates of the later defined genus *Herbaspirillum* were initially thought to be a new *Azospirillum* species (Baldani et al., 1984). However, RNA-RNA hybridization experiments showed no close relatedness with *Azospirillum* spp. or *Aquaspirillum itersonii* (Falk et al., 1986). The first species of the newly defined genus *Herbaspirillum*, *Herbaspirillum seropedicae* (Baldani et al., 1986), was named after the location of the EMBRAPA National Center for Agrobiology (CNPAB) in Seropedica, Rio de Janeiro, Brazil. The genus *Herbaspirillum* was extended with [*Pseudomonas*] *rubrisubalbicans*, causative agent of "mottled strip disease" in some susceptible sugar-cane varieties, because DNA-rDNA and DNA-DNA reassociation hybridization studies showed a high degree of DNA similarity (Gillis et al., 1990; Baldani et al., 1992). Additional physiological and biochemical features, including the ability to fix nitrogen, confirmed the reclassification as *Herbaspirillum rubrisubalbicans* (Baldani et al., 1996). A group of clinical isolates (EF group 1) had to be included in the genus *Herbaspirillum* as "species 3" because of its molecular and overall physiological relatedness. However, members of *Herbaspirillum* species 3 do not exhibit nitrogen-fixing ability. More recently, several new species of *Herbaspirillum* were isolated from diverse plants like *Miscanthus sinensis* and *Pennisetum purpureum* (*H. frisingense*; Kirchhof et al., 2001) and nodules of *Phaseolus* (*H. lusitanum*; Valverde et al., 2003). On the basis of molecular relatedness, a group of bacteria having the ability to efficiently degrade chlorophenols was also included in the genus *Herbaspirillum* as *Herbaspirillum chlorophenolicum* (Im et al., 2004). Although most of the bacteria in the genus *Herbaspirillum* are N_2-fixing bacteria colonizing diverse plants endophytically (Döbereiner, 1992; Döbereiner et al., 1993), clinical and environmental isolates belong to this genus, too. This resembles the situation in other species of the Betaproteobacteria, where plant-associated or even symbiotic diazotrophs, opportunistic pathogens, and potent degraders of pollutants belong to the same genera like *Burkholderia* (Coenye and Vandamme, 2003), *Ralstonia* (Chen et al., 2001) and *Azoarcus* (Reinhold-Hurek and Hurek, 2000).

Taxonomy Aspects

Herbaspirillum spp. are members of the Betaproteobacteria which include many plant-associated bacteria such as the above-mentioned genera—*Azoarcus*, *Burkholderia* or *Ralstonia*. According to results based on DNA or RNA analyses, the genus *Herbaspirillum* belongs to the RNA superfamiliy III (De Smedt et al., 1980). DNA and RNA similarity studies clearly separate *Herbaspirillum* spp. from other betaproteobacterial genera and demonstrate a very high genomic DNA similarity in each of the *Herbaspirillum* spp.

Using the 16S-rDNA-based molecular phylogenetic approach the now known five species of *Herbaspirillum* form a close cluster within the Betaproteobacteria. The phylogenetic tree (Fig. 1) illustrates the position of the *Herbaspirillum* spp. and its closest relatives in the Betaproteobacteria. The tree was constructed by a maximum likelihood analysis, and the topology was confirmed by using a distance and maximum parsimony analysis. The 16S rDNA sequence similarity values within the genus *Herbaspirillum* are 98.5–99.4% and were clearly distinct from those of the next nearest relatives, i.e., the *Ultramicrobacterium* strains D-6 and ND5 (Iizuka et al., 1998) with 95.8–97.3% sequence similarity as well as *Janthinobacterium lividum* and *Oxalobacter formigens* with 95.4–96.2% and 94.6–95.4% (Sievers et al., 1998) sequence similarity, respectively. Within the different *Herbaspirillum* species, the 16S rDNA sequence similarities are very high. For example, *Herbaspirillum frisingense*, comprising isolates from different fiber plant tissues from Germany and Brazil, forms a tight cluster with 16S rRNA similarities of 98.9–99.4% (Kirchhof et al., 2001). Compared to the

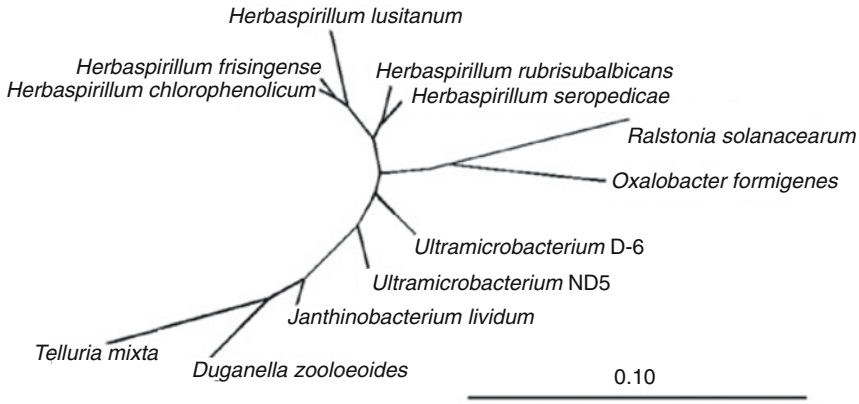

Fig. 1. 16S rDNA phylogenetic tree of *Herbaspirillum* (consensus tree). For the calculation of the phylogenetic tree, almost complete 16S rDNA sequences of the validly named *Herbaspirillum* spp. and most closely related members of the Betaproteobacteria were used. Only sequence positions which are represented in more than 50% of the members of the shown Betaproteobacteria were used for the calculation. The phylogenetic tree is based on "maximum likelihood" analysis and the topology of the tree was checked with "maximum parsimony" and "distance matrix" analyses.

Table 1. 16S rRNA-targeted oligonucleotide probes for fluorescence in situ hybridization (FISH-analysis) of *Herbaspirillum* spp.

Probe	Sequence 5′-3 position	Target	Specificity	Formamide (%)
HERB 68	AGCAAGCTCCTATGCTGC	68–85	Genus *Herbaspirillum*	35
HERB 1432	CGGTTAGGCTACCCACTT	1432–1449	Genus *Herbaspirillum*	35
Hsero 445	GCCAAAACCGTTTCTTCC	445–462	*H. seropedicae*	35
Hrubri 445	GCTACCACCGTTTCTTCG	445–462	*H. rubrisubalbicans*	60
Hfris 445	TCCAGAACCGTTTCTTCC	445–462	*H. frisingense*	50

From Kirchhof et al. 2001.

type strain of *Herbaspirillum seropedicae* (LMG 6513^T), *H. frisingense* strains have 16S rDNA sequence similarities of 98.7–99.1%. The high 16S rDNA similarity of 98.5–99.4% within the genus *Herbaspirillum* does not conclusively imply the differentiation of distinct species (Stackebrandt and Goebel, 1994). However, genomic DNA-DNA hybridization clearly allowed the differentiation, because the percentage of chromosomal DNA reassociation was 11% and 34% between *H. seropedicae* LMG 6513^T, *H. frisingense* [DSM13128]^T and *H. rubrisubalbicans* [LMG 2286]^T, respectively. Within the different species, the DNA-DNA hybridization values are 60–100% and the overall DNA G+C content (mol%) is 61–65% in all *Herbaspirillum* spp.

On the basis of the complete 16S rDNA sequences and the use of ARB-software for sequence analysis (Ludwig et al., 2004), it was possible to create a set of phylogenetic oligonucleotide probes on the genus and the species level (Hartmann et al., 2000; Kirchhof et al., 2001; M. Schmid and M. Rothballer, unpublished observation; Table 1). Using these probes, *H. seropedicae*, *H. rubrisubalbicans*, *H. frisingense* and *H. lusitanum* cells can easily be identified using the fluorescence in situ hybridization (FISH) technique (Amann et al., 1990; Wagner et al., 2003). In addition, 23S rDNA-directed oligonucleotide probes HS and HR complementary to a highly variable stretch of helix (position 55 to 59) of the 23S rRNA of *H. serpedicae* and *H. rubrisubalbicans* were developed (Kirchhof et al., 1997b). These probes were used for radioactive or nonradioactive filter hybridization in the identification of newly obtained isolates (Kirchhof et al., 1997a) but are not suitable for FISH analysis. They cannot be used, e.g., for specific differentiation between *H. rubrisubalbicans* and *H. frisingense*.

Polymerase chain reaction (PCR)-fingerprinting can be applied for the differentiation of DNA at the level of strains (Rademaker and De Bruijn, 1997). The clonal diversity of a variety of *Herbaspirillum* isolates was analyzed with different randomly amplified polymorphic DNA (RAPD) primers (Soares-Ramos et al., 2003) and primers directed to sequences derived from eukaryotic LINEs (long interspersed nuclear elements) conserved in all cells (Smida et al., 1996; Kirchhof et al., 2001; Valverde et al., 2003). The separation power was higher than the one obtained or achievable by amplified rDNA

restriction analysis (ARDRA) using four endonucleases *Alu*I, *Hae*III, *Hinf*I and *Rsa*I (Cruz et al., 2001). When different *Herbaspirillum* species were compared, RAPD- and LINE-analysis-derived banding patterns confirmed the different species, and it became additionally apparent that isolates of the same species (e.g., *H. frisingense*), originating from different plants, exhibit a different, although related, genomic fingerprint (Kirchhof et al., 2001). Isolates from roots, stems and leaves of banana formed a separate group (Soares-Ramos et al., 2003) which may even represent a new species. These findings indicate that the genetic diversity of plant-associated bacterial strains can be correlated with their plant origin (McArthur et al., 1988), reflecting a possible coevolution of plant endophytic bacteria with their hosts.

Habitats and Ecology

The origin of bacteria of the genus *Herbaspirillum* was mostly plant material, and the isolated strains showed the ability to fix nitrogen. In many cases, plant-associated *Herbaspirillum* spp. were found in apoplastic (Olivares et al., 1997; Elbeltagy et al., 2001) or intracellular locations (James et al., 1997; Olivares et al., 1997). When associated with plants, either as an asymptomatic bacterium or as a causal agent of mild disease, *Herbaspirillum* species have been found in species of the family Gramineae, like rice, wild rice (*Oryza officinalis*), *Sorghum bicolor*, *Miscanthus sinensis*, and *Pennisetum purpureum* (Baldani et al., 1996; Elbeltagy et al., 2000; Kirchhof et al., 2001). They are also associated with dicotyledoneous plants and could be isolated from root nodules of the legume *Phaseolus vulgaris* (Valverde et al., 2003) and roots as well as stems of different cultivars of banana (*Musa* spp.) and pineapple (*Ananas comosus* (L.) Merril; Weber et al., 1999; Weber et al., 2001; Cruz et al., 2001).

Some strains of *H. rubrisubalbicans* are mild pathogens of some susceptible sugar-cane varieties causing "mottled stripe disease"; they occur mainly in crops highly fertilized with nitrogen. However, all commercially used sugar-cane varieties in Brazil are resistant to this disease and *H. rubrisubalbicans* and *H. seropedicae* did not produce any characteristic symptoms when artificially inoculated into leaves by injection (Olivares et al., 1997). In addition, strains of *H. seropedicae* and *H. rubrisubalbicans* cause "red stripe disease" in *Pennisetum purpureum* as well as in *Sorghum bicolor* although symptoms are very mild in *Sorghum* leaves inoculated artificially (Pimentel et al., 1991; Olivares et al., 1997). No symptoms were observed in maize plants inoculated with *H. seropedicae* and *H. rubrisubalbicans*. In addition, no visible pathologic symptoms were apparent when *H. frisingense* was inoculated to *Miscanthus sinensis* seedlings (Eckert, 2003). In a survey to characterize the rhizobial community in nodules of *Phaseolus vulgaris*, isolates of a novel *Herbaspirillum* species, *H. lusitanum* ([LMG21710])^{T}, were obtained recently (Valverde et al., 2003). These bacteria were demonstrated to be infectious to *P. vulgaris* roots under axenic conditions, confirming the endophytic character of *H. lusitanum*.

In contrast to these plant associated *Herbaspirillum* spp., bacterial isolates (EF-group 1; Falsen, 1996) from different clinical specimens were grouped as *Herbaspirillum* sp. 3 (Gillis et al., 1991). Finally, an isolate from a 4-chlorophenol contaminated soil sediment was validly named "*H. chlorophenolicum* ([CPW301])^{T}" ([KCTC12096]^{T}; Im et al., 2004). This isolate was originally named "*Comamonas testosteroni*", collected from a stream near an industrial region in Cheongju, Korea, and selectively enriched using 4-chlorophenol as the sole carbon and energy source (Bae et al., 1996).

The majority of *H. seropedicae* and *H. rubrisubalbicans* isolates has been found in plants of tropical areas in numbers varying from 102 to 107 cells per g of fresh plant tissue (Table 2). Strains of *H. seropedicae* were first isolated from washed and surface sterilized roots of maize, sorghum and rice grown in two different soils in Rio de Janeiro State as well as from maize plants grown in a Cerrado soil in Brasilia, DF, Brazil (Baldani et al., 1986). Only a few isolates were obtained from rhizosphere soil (Baldani et al., 1986). Since *H. seropedicae* could not survive well in soil (Olivares et al. 1996), small root pieces could have been present in the rhizosphere soil used by Baldani et al. (1986).

Herbaspirillum seropedicae is a plant-endophytic bacterium (James and Olivares, 1998; James et al., 2002) infecting and colonizing tissues of rice roots mostly in the intercellular space, the apoblast. Using electron microscope analysis, *H. rubrisubalbican*s was localized in the intercellular space of the xylem and in the substomatal cavities of a mottled stripe susceptible sugar-cane variety, where the bacteria are restricted to microcolonies encapsulated within membranes of plant cell origin (Olivares et al., 1997). *Herbaspirillum seropedicae* and *H. rubrisubalbicans* were localized in the xylem in sugar-cane roots (Olivares et al., 1997) and *H. frisingense* in intercellular spaces of the root cortex and the root vascular tissue of *Miscanthus sinensis* roots (Eckert, 2003).

Table 2. Habitats and sources of isolation of *Herbaspirillum* spp.

Species	Country	References[a]
Herbaspirillum seropedicae		
Roots, stems and leaves of maize, sorghum, rice and sugar cane	Brazil	Baldani et al., 1986 Olivares et al., 1996
Roots of *Echinola crusgalli*, *Pennisetum purpureum*, *Panicum maximum*, *Digitaria decumbens*, *Brachiaria decumbens*, *Melinis minutiflora*	Brazil	Olivares et al., 1996
Stems of cultivated (*Oryza sativa*) and wild rice (*O. officinalis*, *O. barthii*, *O. rufipogon*)	Japan	Elbeltagy et al., 2000
Roots, stems and leaves of banana (*Musa* spp.)	Brazil	Weber et al., 1999, 2001
Herbaspirillum rubrisubalbicans		
Roots, stems and leaves of sugar cane and roots of *Digitaria insularis*	Brazil	Olivares et al., 1996
Roots, stems and leaves of banana and pineapple	Brazil	Weber et al., 1999
Herbaspirillum frisingense		
Roots, stems and leaves of *Miscanthus sinensis, M. sacchariflorus, Spartina pectinata*	Germany	Kirchhof et al., 1997, 2001
Roots and stems of *Pennisetum purpureum*	Brazil	Kirchhof et al., 2001
Herbaspirillum lusitanum		
Root nodules of *Phaseolus vulgaris*	Portugal	Valverde et al., 2003
Herbaspirillum species 3		
Different clinical specimen and infections (EF-group 1a and 1b)	Sweden	Falsen, 1996 Gillis et al., 1991
Herbaspirillum chlorophenolicum		
Contaminated sediment of a stream in an Industrial region in Cheongju	Korea	Bae et al., 1996 Im et al., 2004

[a]These references are representative of the literature in this area.

Isolation Procedures

Isolations of the nitrogen-fixing species *Herbaspirillum seropedicae*, *H. rubrisubalbicans* and *H. frisingense* take advantage of their ability to fix nitrogen under microaerobic conditions, as in the case of other microaerobic nitrogen-fixing bacteria like *Azospirillum* and *Gluconacetobacter* (Döbereiner, 1990). Serial dilutions of macerated root, stem or leave samples are inoculated into serum vials with nitrogen-free semisolid (1.75 g of agar/liter) NFb or JNFb medium (Table 3) and incubated at 32°C for one week (Döbereiner, 1995). In vials which exhibit a fine white pellicle, cells are examined under the microscope for the presence of small curved rods (0.6–0.7 × 4–6 μm). Following a transfer to fresh JNFb semisolid medium and incubation for 24–48 h, cultures are streaked out on solid JNFb medium containing 20 mg of yeast extract per liter and three times the bromothymol blue concentration of the JNFb medium. *Herbaspirillum seropedicae* and *H. rubrisubalbicans* form small moist white colonies with a green or dark blue center, in contrast to white colonies of *Azospirillum lipoferum* and *A. brasilense*. In the case of *H. frisingense*, the colored center of the colonies is not as highly marked as in the typical colonies of *H. seropedicae* and *H. rubrisubalbicans*. For final purification, single colonies are transferred to JNFb semisolid medium and cells from the typical pellicle are streaked onto moist BMS agar plates. Moist, smooth and small brownish colonies develop in the case of *H. seropedicae* and *H. rubrisubalbicans* (Baldani et al., 2003).

The original isolation of *H. lusitanum* (Valverde et al., 2003) was performed according to Vincent (1970) using YMA agar (Bergersen, 1961), because it was intended to isolate *Rhizobia*. On these plates, the colonies of *H. lusitanum* were mucoid, circular convex, white, slightly translucent, and usually 1–2 mm in diameter after two days at 28°C.

Herbaspirillum chlorophenolicum (Im et al., 2004), formerly *Comamonas testosteroni*, was isolated from a contaminated soil sediment near a stream in an industrial region of Korea (Bae et al., 1996) using 4-chlorophenol as the sole carbon and energy source.

Preservation of Cultures

Strains can be preserved in glycerol at –20°C or –80°C by mixing equal volumes of sterilized glycerol and washed, resuspended cells from a 48-h old culture grown in liquid JNFb medium (containing 20 mg of yeast extract and 5 mM ammonium chloride or sodium glutamate). Strains can also be kept lyophilized for many years. Cells grown on slant JNFb medium with D-glucose instead of malic acid for 48–72 h at 30°C are suspended in 2 ml of a 10% sucrose solution and

Table 3. Media used for the isolation and cultivation of diazotrophic *Herbaspirillum* spp.

Ingredient (per liter)	Semisolid NFb medium[a]	Semisolid JNFb medium[a]	Potato agar[b]
DL-Malic acid	5.0 g	5.0 g	2.5 g
Sucrose	None	None	2.5 g
K_2HPO_4	0.5 g	0.13 g	None
KH_2PO_4	None	None	None
$MgSO_4 \cdot 7H_2O$	0.2 g	0.25 g	None
NaCl	0.1 g	1.20 g	None
$CaCl_2 \cdot 2H_2O$	0.02 g	0.25 g	None
$Na_2MoO_4 \cdot 2H_2O$	None	None	None
Na_2SO_4	None	2.40 g	None
$NaHCO_3$	None	0.22 g	None
Na_2CO_3	None	0.09 g	None
K_2SO_4	None	0.17 g	None
Minor element solution[c]	2 ml	2 ml	2 ml
Bromthymol blue solution, 0.5% in 0.2 N KOH	2 ml	None	None
Fe-EDTA, 1.64%	4 ml	4 ml	None
pH (adjusted with KOH)	6.8	5.8	6.8
Vitamin solution[d]	1 ml	1 ml	1 ml
Agar	1.75 g	1.75 g	15 g

[a]Ingredients should be added to the medium in the stated order. For the cultivation under non-N_2-fixing conditions on solid agar plates ($15\,g \cdot liter^{-1}$) under air, 20 mM NH_4Cl has to be added.
[b]Totally, 200 g fresh potatoes are peeled and cooked for 30 min and filtered through cotton before other ingredients are added.
[c]$CuSO_4 \cdot 5H_2O$, 0.4 g; $ZnSO_4 \cdot 7H_2O$, 0.12 g; H_2BO_3, 1.4 g; $Na_2MoO_4 \cdot 2H_2O$, 1.0 g; $MnSO_4 \cdot H_2O$, 1.5 g; and H_2O, 1000 ml.
[d]Biotin, 10 mg; Pyridoxol-HCl, 20 mg; and H_2O, 100 ml.

Table 4. Discriminative phenotypic characteristics of *Herbaspirillum* spp.

	H. seropedicae	*H. rubrisubalbicans*	*H. frisingense*	*H. lusitanum*	*H. chlorophenolicum*
Assimilation of					
N-Acetyl-D-glucosamine	+	–	+	+	+
meso-Inositol	+	–	–	–	–
L-Rhamnose	+	–	–	+	–
meso-Erythritol	–	+	–	–	nd
Arabinose	+	+	–	+	nd

Symbols: +, present; –, absent; and nd, not determined.

5% peptone in 100 ml water. Aliquots are distributed into lyophilization ampoules and lyophilized.

Stock cultures can also be maintained on JNFb or BMS agar under a layer of sterilized mineral oil in tubes tightly sealed with rubber caps. Under these conditions, *H. seropedicae* remains viable at room temperature for at least 12 years (Baldani et al., 2003).

Identification

Cells of *Herbaspirillum* spp. exhibit Gram-negative staining. As originally described by Baldani et al. (1986) and Baldani et al. (1996), they generally have a vibroid cell shape, but they are, depending on the growth conditions, spirillum-shaped with a diameter of approximately 0.6–0.7 μm. Cell length depends on the culture medium and varies between 1.5 μm and 5.0 μm. They are very motile, using one to three flagella at one or both poles (Baldani et al., 2003).

The organisms have a strictly respiratory type of metabolism and sugars are oxidized but not fermented. With the exception of *Herbaspirillum* sp. 3 and *H. chlorophenolicum*, herbaspirilla are able to fix atmospheric N_2 under microaerobic conditions. They are oxidase and urease positive, but the catalase is variable or weak. The favored carbon sources are salts of organic acids like malate, pyruvate, succinate and fumarate both for NH_4^+ or N_2-dependent growth. Other carbon sources like glycerol, mannitol, D-glucose and sorbitol are also catabolized. However, sucrose cannot be utilized. Phenotypic characteristics which separate the five validly named *Herbaspirillum* spp. are summarized in Table 4. As shown by Valverde et al. (2003), *Herbaspirillum* spp. also exhibit a unique antibiotic resistance pattern which may also be used for differentiation.

The optimal temperature is 30–34°C and optimal pH, 5.3–8.0. The colonies on JNFb agar plates containing bromothymol blue are smooth and white with blue or green centers after one week of incubation.

Herbaspirillum seropedicae, *H. rubrisubalbicans* and *H. frisingense* can rapidly be identified using 16S rRNA-directed oligonucleotide probes and the fluorescence *in situ* hybridization (FISH) technique (Kirchhof et al., 2001; Table 1; Fig. 1).

Physiology

Herbaspirillum spp. are microaerophilic nitrogen-fixing bacteria except the mostly clinical *Herbaspirillum* species 3 and the very recently renamed species *H. chlorophenolicum* (Im et al., 2004). The diazotrophic herbaspirilla form a pellicle below the surface in nitrogen-free semisolid agar because of their microaerobic characteristic. They cannot grow or fix nitrogen in liquid N-free medium under air. However, nitrogenase activity can be detected under air when grown in liquid JNFb medium supplemented with L-glutamate and L-glutamine but not with L-serine, L-alanine or ammonium chloride when the nitrogen source is exhausted from the medium (Klassen et al., 1997). This is in contrast to some species of the genus *Azospirillum*, which can grow and fix nitrogen simultaneously, e.g., on glutamate as sole carbon and nitrogen source (Hartmann et al., 1988). Other nitrogen sources such as L-histidine, L-lysine, L-arginine or the amines methylammonium chloride, tetramethylammonium chloride, and ethylenediamine chloride do not support growth or nitrogen fixation by *H. seropedicae* (Klassen et al., 1997). *Herbaspirillum seropedicae* can assimilate or dissimilate nitrate to nitrite under oxygen limitation, but no nitrate-dependent anaerobic growth or visible gas production from nitrate is observed. However, small amounts of nitrous oxide (N_2O) are detected in the presence of 10% acetylene. Most strains of *H. rubrisubalbicans* also reduce nitrate to nitrite, but denitrification has not been observed. *Herbaspirillum chlorophenolicum* is not able to reduce nitrate to nitrite.

Compounds that can serve diazotrophic *Herbaspirillum* spp. as sole carbon and energy sources for N_2-dependent growth include malate, succinate, citrate, α-ketoglutarate, fumarate, pyruvate, *trans*-aconitate as well as mannitol, glycerol, sorbitol, glucose, galactose, and L-arabinose. *N*-Acetylglucosamine is used as sole carbon source for N_2-dependent growth by *H. seropedicae*, *H. frisingense* and *H. lusitanum* but not by *H. rubrisubalbicans*. In contrast, *meso*-erythritol is only used by *H. rubrisubalbicans* when the mannitol component of YMA medium is replaced by this carbon source and a nitrogen source like ammonium chloride is present in the medium.

Herbaspirillum seropedicae is the most intensively studied *Herbaspirillum* species. Since *H. seropedicae* is a diazotrophic plant growth promoting bacterium with potential for application as "green fertilizer," the studies focus on the nitrogen metabolism, especially the molecular organization and regulation of nitrogen fixation and ammonium assimilation genes and activities. The structural organization and regulation of the nitrogen fixation genes are well known (Machado et al., 1996; Klassen et al., 1999; Pedrosa et al., 2001). Nitrogen fixation in this organism occurs under microaerobic conditions and is tightly regulated by nitrogen compounds both at the level of synthesis and activity. In addition, ammonium causes a rapid and reversible switch-off of nitrogenase activity in *H. seropedicae*, as it does in *Azospirillum brasilense* and *A. lipoferum* (Hartmann et al., 1986; Fu and Burris, 1989). The central regulator of nitrogen control is the NifA protein, the *nif*-specific transcriptional activator in response to the levels of fixed nitrogen and oxygen (Souza et al., 1999). In addition, the general nitrogen control of the cell is regulated by NtrC, which also controls the expression of the *glnA* gene coding for glutamine synthetase, the key enzyme of the high affinity ammonium assimilation pathway (Persuhn et al., 2000; Souza et al., 2000). In contrast to the gamma-proteobacteria *Klebsiella pneumoniae* and *Azotobacter vinelandii*, where the NifL protein forms an inactive complex with the NifA protein in the presence of high levels of ammonium and oxygen, the NifA-protein is directly inactivated in response to increased levels of nitrogen and oxygen in *H. seropedicae* and the alpha-proteobacterium *Azospirillum brasilense* (Souza et al., 1991; Arsène et al., 1996). Although the mechanism of NifA activity control differs in these two groups of bacteria, the signaling pathways leading to the ammonium response have similarities. In strains of *A. brasilense* and *H. seropedicae*, which do not contain NifL, the P_{II} protein—the product of the *glnB* gene—is necessary for the ammonium control of NifA activity (Benelli et al., 1997). The signaling pathway for control of NifA activity by oxygen in rhizobia (*A. brasilense* and *H. seropedicae*) is probably sensed directly by their type of NifA protein (Monteiro et al., 1999). It has been suggested that the oxygen sensitivity of these NifA proteins involves a cysteine motif located at the end of the central domain and a linker region for the C-terminal domain, which resembles an iron-sulfur cluster-binding motif (Fischer et al., 1988). It has recently been demontrated that an alternative iron containing signal transducer for oxygen sensitivity of the NifA activity in *H. seropedicae* involves the Fnr protein, a general transcriptional regulator for the switch from aerobic to anaerobic metabolism

responsive to molecular oxygen (Monteiro et al., 2003). NifA expression is controlled by the general nitrogen regulation Ntr system which, in turn, is controlled by the state of the *glnB* product, the P_{II} protein. In *H. seropedicae*, the *glnA*, *glnB* and *ntrBC* genes have been identified (Benelli et al., 1997), suggesting that an Ntr/P_{II}-dependent signal transducer cascade senses the nitrogen levels in this organism, as it does in *A. brasilense*. A second P_{II}-like protein, called "GlnK" like in enteric bacteria, has been characterized in *H. seropedicae* (Benelli et al., 2002); it is regulated by uridylylation (Benelli et al., 2001).

Using gfp-reporter constructs, the in situ expression of the *nifH*-gene was recently demonstrated in *H. seropedicae* Z67 during the endophytic colonization of different gramineous plants (Roncato-Maccari et al., 2002). Similar results of *in situ nifH*-activity were obtained with *Azoarcus* sp. BH72 colonizing rice roots endophytically (Reinhold-Hurek and Hurek, 1998).

Application

Owing to their ability to fix nitrogen and to produce phytohormones (Bastián et al., 1998; Lambrecht et al., 2000), the diazotrophic *Herbaspirillum* spp. have the potential of plant growth promotion and associative nitrogen fixation (Baldani et al., 1995; Boddey et al., 1995; James, 2000). *Herbaspirillum* spp. are aggressive colonizers of the root interior, establishing themselves not only in the cortex and vascular tissues of roots but also systemically in the whole plant. Using axenic systems of different plants, a significant stimulation of root development due to inoculation by *H. seropedicae* (Baldani et al., 1993) and *H. frisingense* (Eckert, 2003) was demonstrated. Up to now only *Herbaspirillum seropedicae* strains have been applied in field experiments. Pereira et al. (1988) and Baldani et al. (2000) showed significant yield increases of sorghum and rice when inoculated with *H. seropedicae*. Increases of dry weight and grain yield were also observed in rice plants inoculated with several strains of *H. seropedicae* (Döbereiner and Baldani, 1998). Certain aluminum (Al)-tolerant rice varieties were stimulated in growth and nitrogen accumulation because of inoculation with *Herbaspirillum seropedicae* (Gyaneshwar et al., 2002). *Herbaspirillum*-inoculated Al-tolerant varieties (e.g., cv. Moroberekan) showed significantly more $^{15}N_2$ incorporation and higher N-contents than did the Al-sensitive variety IR45. Al-tolerant varieties secrete larger amounts of C in their root exudates, and bacteria colonizing the roots of cv. Moroberekan strongly expressed gusA- and NifH-proteins. Since *Herbaspirillum* spp. are frequently occurring in agricultural soils in the tropics and subtropics, the inoculation effect is sometimes difficult to assess because of the lack of a clear negative control. It is also possible that *Herbaspirillum* spp. are distributed and introduced through the seeds or plant stocks in the field.

Acknowledgment. This chapter is dedicated to the late Dr. Johanna Döbereiner. Her enthusiasm for plant associated nitrogen-fixing bacteria led to the original discovery of *Herbaspirillum* and many other diazotrophs at the EMBRAPA Center for Agrobiological Research in Seropedica, Rio de Janeiro, Brazil.

Literature Cited

Amann, R. I., L. Krumholz, and D. A. Stahl. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative and environmental studies in microbiology. J. Bacteriol. 172:762–770.

Arsène, F., P. A. Kaminski, and C. Elmerich. 1996. Modulation of NifA activity by PII in Azospirillum brasilense Sp7: Evidence for a regulatory role of the NifA N-terminal domain. J. Bacteriol. 178:4830–4838.

Bae, H.-S., J. M. Lee, Y. B. Kim, and S. T. Lee. 1996. Biodegradation of the mixtures of 4-chlorophenol and phenol by Comamonas testosteroni CPW301. Biodegradation 7:463–469.

Baldani, J. I., V. L. D. Baldani, M. J. A. M. Sampaio, and J. Döbereiner. 1984. A fourth Azospirillum species from cereal roots. An. Acad. Brasil. Cienc. 56:365.

Baldani, J. I., V. L. D. Baldani, L. Seldin, and J. Döbereiner. 1986. Characterization of Herbaspirillum seropedicae gen. nov., sp. nov., a root associated nitrogen-fixing bacterium. Int. J. Syst. Bacteriol. 36:86–93.

Baldani, V. L. D., J. I. Baldani, F. L. Olivares, and J. Döbereiner. 1992. Identification and ecology of Herbaspirillum seropedicae and the closely related [Pseudomonas] rubrisubalbicans. Symbiosis 13:65–73.

Baldani, V. L. D., E. K. James, J. I. Baldani, and J. Döbereiner. 1993. Colonization of rice by the nitrogen-fixing Herbaspirillum spp. and Azospirillum brasilense. *In:* R. Palacios, J. Mora, and W. E. Newton (Eds.) New Horizons in Nitrogen Fixation. Kluwer Academic Publishers. Dordrecht, The Netherlands. 705.

Baldani, V. L. D., F. L. Olivares, and J. Döbereiner. 1995. Selection of Herbaspirillum spp. strains associated with rice seedlings amended with ^{15}N-labeled fertilizer. *In:* International Symposium on Sustainable Agriculture for the Tropics: The Role of Biological Nitrogen Fixation, Angra dos Reis, Brazil.

Baldani, J. I., B. Pot, G. Kirchhof, E. Falsen, V. L. D. Baldani, F. J. Olivares, B. Hoste, K. Kersters, A. Hartmann, M. Gillis, and J. Döbereiner. 1996. Emended description of Herbaspirillum; inclusion of [Pseudomonas] rubricubalbicans, a mild pathogen, as Herbaspirillum comb. nov.; and classification of a group of clinical isolates (EF group 1) as Herbaspirillum species 3. Int. J. Syst. Bacteriol. 46:802–810.

Baldani, V. L. D., J. I. Baldani, J. Döbereiner. 2000. Inoculation of rice plants with the endophytic diazotrophs Herbaspirillum seropedicae and Burkholderia spp. Biol. Fertil. Soils 30:485–489.

Baldani, J. I., V. L. D. Baldani, and J. Döbereiner. 2003. Genus Herbaspirillum. *In:* Bergey's Manual of Determinative Bacteriology.

Bastián, F., A. Cohen, P. Piccoli, V. Luna, R. Baraldi, and R. Bottini. 1998. Production of indole-3-acetic acid and gibberelins A1 and A3 by Acetobacter diazotrophicus and Herbaspirillum seropedicae in chemically-defined culture media. Plant Growth Regul. 24:7–11.

Benelli, E. M., E. M. Souza, S. Funayama, L. U. Rigo, and F. O. Pedrosa. 1997. Evidence for two possible glnB-type genes in Herbaspirillum seropedicae. J. Bacteriol. 179:4623–4626.

Benelli, E. M., M. Buck, E. M. de Souza, M. G. Yates, and F. O. Pedrosa. 2001. Uridylylation of the P_{II} protein from Herbaspirillum seropedicae. Can. J. Microbiol. 47:309–314.

Benelli, E. M., M. Buck, I. Polikarpov, E. M. de Souza, L. M. Cruz, and F. O. Pedrosa. 2002. Herbaspirillum seropedicae signal transduction protein PII is structurally similar to the enteric GlnK. Eur. J. Biochem. 269:3296–3303.

Bergersen, F. J. 1961. The growth of Rhizobium in synthetic media. Austral. J. Biol. 14:349–360.

Boddey, R. M., O. C. de Oliveira, S. Urquiaga, V. M. Reis, F. L. Olivares, V. L. D. Baldani, and J. Döbereiner. 1995. Biological nitrogen fixation associated with sugar cane and rice: Contributions and prospects for improvement. Plant Soil 174:195–209.

Chen, W.-M., S. Laevens, T. M. Lee, T. Coenye, P. De Vos, M. Mergeay, and P. Vandamme. 2001. Ralstonia taiwanensis sp. nov., isolated from root nodules of Mimosa species and sputum of a cystic fibrosis patient. Int. J. Syst. Evol. Microbiol. 51:1729–1735.

Coenye, T., and P. Vandamme. 2003. Diversity and significance of Burkholderia species occupying diverse ecological niches. Environ. Microbiol. 5:719–729.

Cruz, L. M., E. M. Souza, O. B. Weber, J. I. Baldani, J. Döbereiner, and F. O. Pedrosa. 2001. 16S ribosomal characterization of nitrogen-fixing bacteria isolated from banana (Musa spp.) and pineapple (Ananas comusus (L.) Merril). Appl. Environ. Microbiol. 67:2375–3279.

De Smedt, J., M. Bauwens, R. Tytgat, and J. De Ley. 1980. Intra- and intergeneric similarities of ribosomal ribonucleic acid cistrons of free-living, nitrogen-fixing bacteria. Int. J. Syst. Bacteriol. 30:106–122.

Döbereiner, J. 1990. The genera Azospirillum and Herbaspirillum. *In:* A. Balows, H. G. Trüper, M. Dworkin, and W. Harder (Eds.) The Prokaryotes, 2nd ed. Springer-Verlag. Berlin, Germany. 2236–2253.

Döbereiner, J. 1992. History and new perspectives of diazotrophs in association with non-leguminous plants. Symbiosis 13:1–13.

Döbereiner, J., V. M. Reis, M. A. Paula, and F. L. Olivares. 1993. Endophytic diazotrophs in sugar cane, tuber plants and cereals. *In:* R. Palaciaos, J. Mora, and W. E. Newton (Eds.) New Horizons in Nitrogen Fixation. Kluwer Academic Publishers. Dordrecht, The Netherlands. 671–676.

Döbereiner, J. 1995. Isolation and identification of aerobic nitrogen-fixing bacteria from soil and plants. *In:* K. Alef and P. Nannipieri (Eds.) Methods in Applied Soil Microbiology and Biochemistry. Academic Press. London, UK. 134–141.

Döbereiner, J., and V. L. D. Baldani. 1998. Biological nitrogen fixation by endophytic diazotrophs in non-leguminous crops in the tropics. *In:* K. A. Malik, S. Mirza, and J. K. Ladha (Eds.) Nitrogen Fixation with Non-legumes. Kluwer Academic Publishers. Dordrecht, The Netherlands. 3–7.

Eckert, B. 2003. Isolation, identification and localisation of diazotrophic bacteria from C4-plant Miscanthus [doctoral thesis]. Faculty of Biology, Ludwig-Maximilian-University. Munich, Germany.

Elbeltagy, A., K. Nishioka, H. Suzuki, T. Sato, Y. Sato, H. Morisaki, H. Mitsui, and K. Minamisawa. 2000. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. Soil Sci. Plant Nutr. 46:617–629.

Elbeltagy, A., K. Nishioka, T. Sato, H. Suzuki, B. Ye, T. Hamada, T. Isawa, H. Mitsui, and K. Minamisawa. 2001. Endophytic colonization and in planta nitrogen fixation by a Herbaspirillum sp. isolated from wild rice species. Appl. Environ. Microbiol. 67:5285–5293.

Falk, E. C., J. L. Johnson, V. L. D. Baldani, J. Döbereiner, and N. R. Krieg. 1986. Deoxyribonucleic and ribonucleic acid homology studies of the genera Azospirillum and Conglomeromonas. Int. J. Syst. Bacteriol. 36:80–85.

Falsen, E. 1996. Catalogue of strains. *In:* CCUG Culture Collection, 5th ed. University of Göteborg. Göteborg, Sweden.

Fischer, H.-M., T. Bruderer, and H. Hennecke. 1988. Essential and non-essential domains in the Bradyrhizobium japonicum NifA protein: Identification of indispensable cystein residues potentially involved in redox reactivity and/or metal binding. Nucleic Acids Res. 16:2207–2224.

Fu, H., and R. H. Burris. 1989. Ammonium inhibition of nitrogense activity in Herbaspirillum seropedicae. J. Bacteriol. 171:3168–3175.

Gillis, M., J. Döbereiner, B. Pot, M. Goor, E. Falsen, B. Hoste, B. Reinhold, and K. Kersters. 1990. Taxonomic relationships between [Pseudomonas] rubrisubalbicans, some clinical isolates (EF group 1), Herbaspirillum seropedicae and [Aquaspirillum] autotrophicum. *In:* M. Polsinelli, R. Materassi, and M. Vincenzini (Eds.) Nitrogen Fixation. Kluwer Academic Publishers. Dordrecht, The Netherlands. 293–294.

Gyaneshwar, P., E. K. James, P. M. Reddy, B. Reinhold-Hurek, and J. K. Ladha. 2002. Herbaspirillum colonization increases growth and nitrogen accumulation in aluminium tolerant rice varieties. New Phytol. 154:131–146.

Hartmann, A., H. Fu, and R. H. Burris. 1986. Regulation of nitrogenase activity by ammonium chloride in Azospirillum spp. J. Bacteriol. 165:864–870.

Hartmann, A., H. Fu, and R. H. Burris. 1988. Influence of amino acids on nitrogen fixation activity and growth of Azospirillum spp.. Appl. Environ. Microbiol. 54:87–93.

Hartmann, A., M. Stoffels, B. Eckert, G. Kirchhof, and M. Schloter. 2000. Analysis of the presence and diversity of diazotrophic endophytes. *In:* E. Triplett (Ed.) Prokaryotic Nitrogen Fixation: A Model System for Analysis of a Biological Process. Horizon Scientific Press. Wymondham, UK. 727–736.

Iizuka, T., S. Yamanaka, T. Nishiyama, and A. Hiraishi. 1998. Isolation and phylogenetic analysis of aerobic copiotrophic ultramicrobacteria from urban soil. J. Gen Appl. Microbiol. 44:75–84.

Im, W.-T., H.-S. Bae, A. Yokota, and S. T. Lee. 2004. Herbaspirillum chlorophenolicum sp. nov., a 4-

chlorophenol-degrading bacterium. Int. J. Syst. Evol. Microbiol. 54:851–855.

James, E. K., F. L. Olivares, J. I. Baldani, and J. Döbereiner. 1997. Herbaspirillum, an endophytic diazotroph colonizing vascular tissue in leaves of Sorghum bicolor L. Moench. J. Exp. Bot. 48:785–797.

James, E. K., and F. L. Olivares. 1998. Infection and colonization of sugar-cane and other gramineous plants by endophytic diazotrophs. Crit. Rev. Plant Sci. 17:77–119.

James, E. K. 2000. Nitrogen fixation in endophytic and associative symbiosis. Field Crop Res. 65:197–209.

James, E. K., P. Gyaneshwar, P. N. Mathan, W. L. Barraquio, P. M. Reddy, P. P. Iannetta, F. L. Olivares, and J. K. Ladha. 2002. Infection and colonization of rice seedlings by the plant growth-promoting bacterium Herbaspirillum seropedicae Z67. Molec. Plant-Microbe Interact. 15:894–906.

Kirchhof, G., V. M. Reis, J. I. Baldani, B. Eckert, J. Döbereiner, and A. Hartmann. 1997a. Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. Plant Soil 194:45–55.

Kirchhof, G., M. Schloter, B. Aßmus, and A. Hartmann. 1997b. Molecular microbial ecology approaches applied to diazotrophs associated with non-legumes. Soil Biol. Biochem. 29:853–862.

Kirchhof, G., B. Eckert, M. Stoffels, J. I. Baldani, V. M. Reis, and A. Hartmann. 2001. Herbaspirillum frisingense sp. nov., a new nitrogen-fixing bacterial species that occurs in C4-fibre plants. Int. J. Syst. Evol. Microbiol. 51:157–168.

Klassen, G., F. O. Pedrosa, E. M. Souza, S. Funayama, and L. U. Rigo. 1997. Effect of nitrogen compounds on nitrogenase activity in Herbaspirillum seropedicae SMR1. Can. J. Microbiol. 43:994–891.

Klassen, G., F. O. Pedrosa, E. M. Souza, M. G. Yates, and L. U. Rigo. 1999. Sequence and functional analysis of the nifNX orf1 orf2 operon from Herbaspirillum seropedicae. FEMS Microbiol. Lett. 181:165–170.

Lambrecht, M., Y. Okon, A. Vande Broek, and J. Vanderleyden. 2000. Indole-3-acetic acid: A reciprocal signalling molecule in bacteria-plant interactions. Trends Microbiol. 8:298–300.

Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Förster, I. Brettske, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. König, T. Liss, R. Lüßmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, and K.-H. Schleifer. 2004. ARB, a software environment for sequence data. Nucleic Acids Res. 32:1–9.

Machado, I. M., M. G. Yates, H. B. Machado, E. M. Souza, and F. O. Pedrosa. 1996. Cloning and sequencing of the nitrogenase structural genes nifHDK of Herbaspirillum seopedicae. Braz. J. Med. Biol. Res. 29:1599–1602.

McArthur, J. V., D. A. Kovacic, and M. H. Smith. 1988. Genetic diversity in natural populations of a soil bacterium across a landscape gradient. Proc. Natl. Acad. Sci. USA 85:9621–9624.

Monteiro, R. A., E. M. de Souza, S. Funayama, M. G. Yates, F. O. Pedrosa, and L. S. Chubatsu. 1999. Expression and functional analysis of an N-truncated nifA protein of Herbaspirillum seropedicae. FEBS Lett. 447:283–286.

Monteiro, R. A., E. M. de Souza, M. G. Yates, F. O. Pedrosa, L. S. Chubatsu. 2003. Fnr is involved in oxygen control of Herbaspirillum seropedicae n-truncated nifA protein activity in Escherichia coli. Appl. Environ. Microbiol. 69:1527–1531.

Olivares, F. L., V. L. D. Baldani, V. M. Reis, J. I. Baldani, and J. Döbereiner. 1996. Occurrence of the endophytic diazotrophs Herbaspirillum spp. in roots, stems, leaves, predominantly of Gramineae. Biol. Fertil. Soils 21:197–200.

Olivares, F. L., E. K. James, J. I. Baldani, and J. Döbereiner. 1997. Infection of mottled stripe disease-susceptible and resistant sugar cane varieties by the endophytic diazotroph Herbaspirillum. New Phytol. 135:723–737.

Pedrosa, F. O., E. M. Benelli, M. G. Yates, R. Wassem, R. A. Monteiro, G. Klassen, M. B. R. Steffens, E. M. de Souza, L. S. Chubatsu, and L. U. Rigo. 2001. Recent developments in the structutal organization and regulation of nitrogen fixation genes in Herbaspirillum seropedicae. J. Biotechnol. 91:189–195.

Pereira, J. A. R., V. A. Cavalcante, J. I. Baldani, and J. Döbereiner. 1988. Field inoculation of Sorgum and rice with Azospirillum spp. and Herbaspirillum seropedicae. Plant Soil 10:269–274.

Persuhn, D. C., M. B. R. Steffens, F. O. Pedrosa, E. M. de Souza, M. G. Yates, and L. U. Rigo. 2000. The transcriptional activator NtrC controls the expression and activity of glutamine synthetase in Herbaspirillum seropediacae. FEMS Microbiol. Lett. 192:217–221.

Pimentel, J. P., F. L. Olivares, R. M. Pitard, S. Urquiaga, F. Akiba, and J. Döbereiner. 1991. Dinitrogen fixation and infection of grass leaves by [Pseudomonas] rubrisubalbicans and Herbaspirillum seropedicae. Plant Soil 137:61–65.

Rademaker, J. L. W., and F. J. De Brujin. 1997. Characterization and classification of microbes by REP-PCR genomic fingerprinting and computer-assisted pattern analysis. *In:* G. Caetano-Anolles and P. M. Gresshoff (Eds.) NA Markers: Protocols, Applications and Overviews. Wiley. Chichester, UK.

Reinhold-Hurek, B., and T. Hurek. 1998. Life in grasses: Diazotrophic endophytes. Trends Microbiol. 6:139–144.

Reinhold-Hurek, B., and T. Hurek. 2000. Reassessment of the taxonomic structure of the diazotrophic genus Azoarcus sensu lato and description of three new genera and new species, Azovibrio restrictus gen. nov., sp. nov., Azospira oryzae gen. nov., sp. nov. and Azonexus fungiphilus gen. nov., sp. nov. Int. J. Syst. Evol. Microbiol. 50:649–659.

Roncato-Maccari, L. D. B., H. J. O. Ramos, F. O. Pedrosa, Y. Alquini, L. S. Chubatsu, R. U. Rigo, M. B. R. Steffens, and E. M. Souza. 2002. Endophytic Herbaspirillum seropedicae expresses the nifH gene in diverse gramineous plants. *In:* J. Vanderleyden (Ed.) Proceedings of the 9th International Symposium on Nitrogen Fixation with Non-legumes. Katholieke Universiteit Leuven and Centre of Microbial and Plant Genetics. Leuven, Belgium. 91.

Sievers, M., H.-G. Schlegel, J. Caballero-Melado, J. Döbereiner, and W. Ludwig. 1998. Phylogenetic identification of two major nitrogen-fixing bacteria associated with sugarcane. Syst. Appl. Microbiol. 21:505–508.

Smida, J., S. Leibhard, A. M. Nickel, F. Eckardt-Schupp, and L. Hieber. 1996. Application of repetitive sequence-based PCR (Inter-LINE PCR) for the analysis of genomic rearrangements and for the genome characterization on different taxonomic levels. Genet. Anal. Biomolec. Engin. 13:95–98.

Soares-Ramos, J. R. L., H. J. O. Ramos, L. M. Cruz, L. S. Chubatsu, F. O. Pedrosa, L. U. Rigo, and E. M. Souza.

2003. Comparative molecular analysis of Herbaspirillum strains by RAPD, RFLP, and 16S rDNA sequencing. Gen. Molec. Biol. 26:537–543.

Souza, E. M., S. Funayama, L. U. Rigo, M. G. Yates, and F. O. Pedrosa. 1991. Sequence and structural organization of a nifA-like gene and part of a nifB-like gene of Herbaspirillum seropedicae strain Z78. Gen. Microbiol. 137:1511–1522.

Souza, E. M., F. O. Pedrosa, M. Drummond, L. U. Rigo, and M. G. Yates. 1999. Control of Herbaspirillum seropecicae NifA activity by ammonium ions and oxygen. J. Bacteriol. 181:681–684.

Souza, E. M., F. O. Pedrosa, L. U. Rigo, H. B. Machado, and M. G. Yates. 2000. Expression of the nifA gene of Herbaspirillum seropedicae: Role of the NtrC and NifA binding sites and of the -24/-12 promotor element. Microbiology 146:1407–1418.

Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. 44:846–849.

Valverde, A., E. Velázquez, C. Gutiérrez, E. Cervantes, A. Ventosa, and J.-M. Igual. 2003. Herbaspirillum lusitanum sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of Phaseolus vulgaris. Int. J. Syst. Evol. Microbiol. 53:1979–1983.

Vincent, J. M. 1970. A Manual for the Practical Study of Root-nodule Bacteria. Blackwell Scientific Publications. IBP Handbook 15.

Wagner, M., M. Horn, and M. Daims. 2003. Fluorescence in situ hybridization for the identification and characterization of prokaryotes. Curr. Opin. Microbiol. 6:302–309.

Weber, O. B., V. L. D. Baldani, K. R. S. Teixeira, G. Kirchhof, J. I. Baldani, and J. Döbereiner. 1999. Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. Plant Soil 210:103–113.

Weber, O. B., L. M. Cruz, J. I. Baldani, and J. Döbereiner. 2001. Herbaspirillum-like bacteria in banana (Musa sp.) plants. Brazil. J. Microbiol. 32:201–205.