# Plant-microbe interactions between maize (*Zea mays* L.) and endophytic microrganisms observed by Scanning Electron Microscopy

**Alexandre Oliveira de Souza[1], João Alencar Pamphile[2]*, Carmem Lúcia de Mello Sartori Cardoso da Rocha[2] and João Lúcio Azevedo[1]**

*[1]Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, PO BOX 83, 13400-970, Piracicaba, São Paulo, Brasil. [2]Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, 87020-900, Maringá, Paraná, Brasil. *Author for correspondence. e-mail: japamphile@uem.br*

**ABSTRACT.** During the last few years endophytes occurrence has been constantly reported in healthy, symptomless leaves, suggesting that this microrganisms can colonize plant organs without causing apparent disease symptoms. The purpose of this study was to observe plant-microbe interactions between maize (*Zea mays* L.) and endophytic bacteria into the leaf tissue by scanning electron microscopy with application of an osmium fixation technique. It was possible to observe intracellular space of maize leaves infected with endophytic bacteria by this technique. The three-dimensional configuration and arrangement of endophyte colonies inside leaf tissue of host plant were visible.

**Key words:** endophytic, bacteria, maize, scanning electron microscopy.

**RESUMO. Interações planta-micróbio entre o milho (*Zea mays* L.) e microrganismos endofíticos observados por microscopia eletrônica de varredura.** Durante os últimos anos, a ocorrência de endófitos tem sido constantemente reportada em folhas saudáveis, assintomáticas, sugerindo que esses microrganismos podem colonizar órgãos de plantas sem causarem sintomas aparentes de doenças. O objetivo deste trabalho foi o de observar as interações planta-micróbio entre o milho (*Zea mays* L.) e bactérias endofíticas no tecido das folhas por microscopia eletrônica de varredura com a aplicação de uma técnica de fixação com ósmio. Com o emprego dessa técnica foi possível observar o espaço intercelular das folhas de milho com bactérias endofíticas. A configuração tri-dimensional e o arranjo das colônias endofíticas nos tecidos das folhas da planta hospedeira foram visíveis.

**Palavras-chave:** endofíticos, bactérias, milho, microscopia eletrônica de varredura.

## Introduction

Endophytes microrganisms, usually bacteria and fungi, live inside healthy plant tissue. Endophytic microorganisms have been isolated in maize (McInroy and Kloepper, 1991; Fisher *et al.*, 1992; Döbereiner *et al.*, 1995; Pamphile and Azevedo, 2002). Evidence for the presence of endophytic bacteria in maize leaves has so far been provided mainly by isolation of the bacteria after surface sterilization of the tissue (McInroy and Kloepper, 1991; Fisher *et al.*, 1992; Döbereiner *et al.*, 1995). However, intracellular mechanisms of infection and colonization of plant tissues by endophytic microrganisms are poorly known. At the cytological level only a few investigations have tried to characterize endophytic bacterial infection and colonization (Döbereiner, 1992; Dong *et al.*, 1994; James *et al.*, 1994; Döbereiner *et al.*, 1995; Taechowisan *et al.*, 2003). Histological studies have been carried out mainly on endophytic bacteria associated with biological nitrogen fixation. Into the present report, we describe intracellular arrangement of endophytic bacteria in leaves of maize plant host using an osmium-fixation technique for scanning electron microscopy (SEM).

## Material and methods

Maize symptomless leaves were used. Small pieces of leaf tissue (about 2 x 6mm) were surface sterilized as described by Pereira *et al*. (1993), and

inoculated for 24h at 37ºC on Petri dishes containing NA medium. After this period, leaf segments were first fixed at 20ºC for 1h in buffered solution containing: 2% glutaraldeyde, 2% paraformaldeide, 0,05 M cacodylate buffer, and 0,001 M $CaCl_2$. After three times rinsing with 0,05 M cacodylate buffer (10 minutes for each rinsing), the specimens were added to a solution containing equal amounts of 0,1 M cacodylate buffer and 2% $OsO_4$ solution buffered at pH 7,2 with 0,1 M cacodylate buffer. After three times rinsing with a distilled water, specimens were successively immersed in a graded acetone series for dehydrate, dried in a critical point dryer (BALZERS-CPD 030) and coated very lightly (60 sec.) with gold in an ion coater (BALZERS-MED 010). Metal-coated specimens were observed in a field emission SEM (ZEISS-DSM940A) at 5 kV.

## Results

Scanning electron microscopy (SEM) showed that the adaxial epidermic and sub-epidermic cells of maize leaves were colonized by different types of rod-shaped endophytic bacteria (ca.2 µm long - Figures 1a-b and 2a-b). The distribution of bacteria cells suggested preferential colonization of the adaxial epidermal cells, where clusters were formed (Figure 2a) or even near the stomata (Figure 1b). These clusters of bacteria appeared to be connected by strands of mucus, sometimes forming extensive networks (Figures 1b, 2b). Isolated cells were found attached to the xylem vessels next to the punctuations (Figure 1a). Identification of bacteria permitted a screening of some of the endophytic genus found in these maize plants as *Bacillus* sp. and *Corynebacterium* sp.

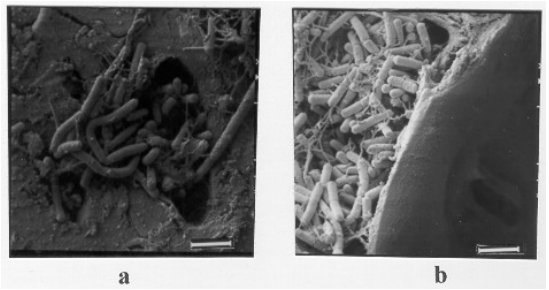


**Figure 1. (a-b)** Scanning electron micrographs (SEM) of maize (*Zea mays* L.) leaf sections. **a.** Longitudinal SEM view of tangencial section of maize xylem vessel. Some isolated bacterial cells are near the punctuations of the vessel. Scale bar = 5 µm. **b.** Transversal section of maize leaf. Cluster of bacteria colonizing an epidermal cell near the stomata. Observe the occurrence of strands of mucous forming a net among the bacteria cells. Scale bar = 5 µm.

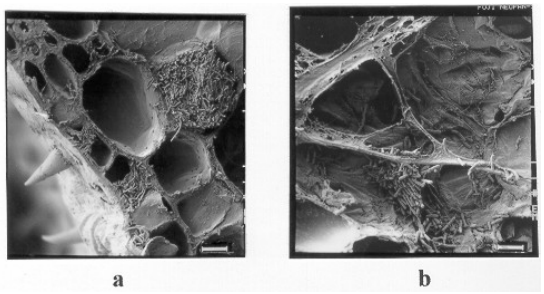


**Figure 2. (a-b)** Scanning electron micrographs (SEM) of transversal sections of maize (*Zea mays* L.) leaves. **a.** Adaxial cross-section of maize leaf, showing unicellular pilli. Bacterial cell clusters are colonizing the epidermal and sub-epidermal cells. Scale bar = 20 µm. **b.** Transversal section of epidermal cells. Bacteria are seen forming a third-dimensional net together the strands of mucous. Scale bar = 10 µm.

## Discussion

The SEM results presented here confirm that infection with endophytic bacteria occurs in maize plants. The SEM investigation was also efficient to reveal the presence of endophytic actinomycetes in *Zingiber officinale* (Taechowisan *et al.*, 2003). In the present study, the bacteria were spread inside host tissue not uniformly, present as clusters inside some epidermal cells, and found as single cells along vascular tissues. This result is in agreement with similar SEM and transmission electron microscopy (TEM) observations of endophytic bacteria on grass tissue (Baldani *et al.*, 1992; Ruppel *et al.*, 1992; Olivares *et al.*, 1995), and imunofluorescent studies. Other reports (Patriquin and Döbereiner, 1978; Döbereiner *et al.*, 1995) have previously established that vascular tissues, for instance, protoxylem and metaxylem vessels, and epidermal cells are important sites for infection and for survival of endophytes, as well as intercellular spaces of mesophyll cells (You *et al.*, 1990). The presence of infection at the adaxial side of the leaves could be interpreted as indirect evidence for colonization of the host after stomatal penetration, as suggested for fungal endophytes (Viret *et al.*, 1993; Viret and Petrini 1994), and for epiphytic bacteria that colonizes senescent leaves of an aquatic macrophyte (Underwood, 1991). Bacilio-Jiménez *et al.* (2001) working with *Oryza sativa* identified, by scanning electron microscopy, endophytic bacteria at the base of secondary roots, between the epidermis and the mucilaginous layer. Most of the studies on infection and distribution of endophytic bacteria on host plant tissues have been developed on associations of diazothrophic endophytes with non leguminous plants. Yang *et al.* (1999) described the occurrency of endophytic

associated diazotrophs intercellularly in rice roots using SEM.

According to James *et al.* (1994), the presence of bacteria in xylem vessels is suggestive that these are a means of transporting bacteria to others parts of the plant, particularly the shoot. Others authors have reported colonization of xylem by endophytic diazothophs (Baldani *et al.*, 1992; Olivares *et al.*, 1995).

Generally, the knowledge generated by studies on plant-microrganism associations, has been of enormous value on the understanding of the role that these microrganisms play into their hosts. Studies on the specificity and establishment of the phenomena of host infection, on the regulatory functions among endophytes and hosts, as well as on the control of this association, during the endophytic phase or not, could be useful to the use of endophytes as a biotechnological material to increase or improve plant production.

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