

# Isolation of endophytic actinomycetes from roots and leaves of maize (*Zea mays* L.)

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## ABSTRACT

*Actinomycetes* were isolated from surface sterilized leaves and roots of maize. A total of 53 isolates were obtained, 31 of them from leaves and 22 from roots. The genus *Microbispora* was the most frequently found followed by the genera *Streptomyces* and *Streptosporangium*. From the isolated actinomycetes, 43.4% showed antimicrobial activity against one or more tested bacteria and yeast.

**Key words:** Endophytes, maize, actinomycetes, *Microbispora*, *Streptomyces*, *Streptosporangium*

## INTRODUCTION

Endophytic microorganisms are those which inhabit the internal part of plants, causing apparently no visible changes to their hosts. Although known since long time, their importance become evident only more recently when it was shown that they play specific roles as for instance, protecting the host-plants against insects and diseases. It has been found also that some endophytic microorganisms can produce valuable pharmaceutical substances of biotechnological interest (Strobel *et al*, 1996; Strobel & Long, 1998). The main roles of endophytic microorganisms are discussed in the reviews of Isaac (1992), Redlin & Carris (1996), Baldani *et al* (1997) and Azevedo (1998).

Actinomycetes are bacteria known to constitute a large part of the rhizosphere microbiota. Their isolation is an important step for screening of new bioactive compounds. Actinomycetes are also found inside plants but, excluding the coryneform bacteria, the only extensively studied species are from the genus *Frankia*, a nitrogen fixing bacteria of non-leguminous plants (Benson & Silvester, 1993) and a few species of the genus *Streptomyces* that are phytopathogens (Bradbury, 1986). Mundt & Hinckle (1976) isolated the genera *Nocardia* and *Streptomyces* from seed and ovules of 27

plant species. Sardi *et al.* (1992) isolated and observed by direct microscope examination endophytic actinomycetes from the roots of 28 plant species from the northwestern Italy. Most of the isolates were classified as *Streptomyces* but the genera *Streptoverticillium*, *Nocardia*, *Micromonospora* and *Streptosporangium* were also found. More recently Matsukuma *et al*, (1995) isolated few actinomycetes from leaves of *Camellia japonica* and citrus, Maitan (1998) observed rare actinomycetes isolated as endophytes of *Solanum lycocarpum* in middlewest Brazil and Matsumoto *et al* (1998) isolated also actinomycetes from fallen leaves and the genus *Microbispora* was the most frequently found, accounting for 44% of all isolates.

The presence of endophytic microorganisms in healthy crops has been demonstrated in maize. Although several fungi and bacteria species were reported, no actinomycetes were isolated (Fisher *et al*, 1992, Souza, 1996; Silva, 1997) and so far, no studies have been undertaken to assess the incidence of endophytic actinomycetes of this important species. The present work was carried out to isolate actinomycetes from roots and leaves of a variety of maize designated Piranão, widely cultivated in Brazil. Also the isolated actinomycetes were screened for antibiotic production against one yeast and three bacteria.

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## MATERIALS AND METHODS

**Plant material** : Seeds from the *Zea mays* variety Piranão were planted in a non fertilized soil, free from insecticides and other chemicals in an experimental field of the "Universidade Federal de Pernambuco ", Recife, located in a tropical area in the northeast Brazil during the summer and plants were sampled 7, 14 and 21 days after the emergence. Only plants exhibiting healthy vegetative growth were used.

**External sterilization of leaves and roots**: Two leaves and two segments of roots from each collected plant were used. Samples were sterilized according to FISHER *et al.* (1992) with modifications (PEREIRA *et al.*, 1993; 1999). They were washed in water following surface sterilization with ethanol 70% for 30 seconds and then treated with sodium hypochloride (3%-5% available chlorine) for 3 minutes. Samples were exhaustively rinsed with sterile water. Sterility checks and reconstruction experiments showed that most or all epiphytic microorganisms were eliminated during this treatment. Each root was divided into pieces of ca. 1 cm and each leaf was divided into fragments of about 1 cm<sup>2</sup>. Root and leaf fragments were aseptically transferred to petri dishes containing starch-casein-agar medium (Kuster & Williams, 1964) and 2.5% water-agar medium. Nystatin and cycloheximide (50µg/ml of each) were added to both media to suppress fungal growth (Williams & Davies, 1965). Plates were incubated at 28<sup>o</sup> C for a maximum of three weeks. Actinomycetes growing on the media were isolated, purified and identified.

**Identification of isolates**: Each isolate was inoculated on specific ISP media (ISP 2, ISP 3, ISP 4 and ISP 5) according to Shirling & Gottlieb (1966) to determine cultural and micromorphological characteristics (Williams *et al.*, 1989; Holt *et al.*, 1994).. The chemical composition of the cell wall from the isolates was also determined (Lechevalier & Lechevalier, 1970; Goodfellow & Minnikin, 1985).

**Antimicrobial activity**: Antimicrobial activity against the bacteria *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis* and the yeast *Candida albicans*, all from the collection of the " Departamento de Antibióticos, Universidade Federal de Pernambuco " were tested on ISP 3 solid medium according to Ichikava *et al.* (1971). Results were scored after 24-48 hours incubation at 37<sup>o</sup> C.

## RESULTS AND DISCUSSION

After a maximum of 21 days incubation, no microbial growth was observed on media inoculated with segments of leaves and roots derived from 7 day-old sampled plants. However, actinomycetes were observed emerging from roots and leaves segments of plants sampled 14 and 21 days after seed germination. The fact that actinomycetes were not isolated from plant tissues until after 7 days does not necessarily mean that they were not present; it could be that sufficient numbers were not present to allow isolation until this time. From a total of 53 isolates, 58.5% were obtained from leaves and the remaining 41.5% from roots. Results giving in Table 1 show that the most frequent genus found was *Microbispora* (33 isolates) followed by *Streptomyces* (6 isolates) and *Streptosporangium* (4 isolates). The remaining 10 isolates never bore reproductive structures and could not be identified. Sardi *et al.*, (1992) found that from 499 endophytic actinomycetes isolated from 28 plant species about 96% *Streptomyces*, 0.2% *Streptosporangium* and no *Microbispora* were reported. Although it can be pointed out that the media used in the present work and also by Sardi *et al.* (1992) are known to select for a few known species which may underestimate total population size, our results agree with those of Matsumoto *et al.* (1998) which found after isolation and identification of actinomycetes from fallen leaves, that the genus *Microbispora* was the most frequently found, accounting for 44% of all isolates.

**Table 1** - Genera and number of endophytic actinomycetes isolated from *Zea mays*

Genera	Number of isolates from		Total
	Leaves	Roots	
<i>Microbispora</i>	21	12	33
<i>Streptomyces</i>	3	3	6
Total	31	22	53

Results giving in Table 2 show that from the 53 isolates tested against bacteria and *C. albicans*, 23 of them (43.4%) presented antimicrobial activity against one or more microorganisms used. Antimicrobial activity was also found in

endophytic actinomycetes (Sardi et al,1992 and Maitan,1998) and thus, actinomycetes may play a role protecting the plant host against pathogenic microorganisms.

**Table 2** - Antimicrobial activity of endophytic actinomycetes isolated from *Zea mays* against bacteria and yeast.

Genera	Isolates*	<i>S.aureus</i> **	<i>M.luteus</i>	<i>B.subtilis</i>	<i>C.albicans</i>
<i>Microbispora</i>	L3,L8,L12,L20,L26.	+	+	+	-
	L29,R36,R39	-	+	-	-
	L13,L14	-	+	+	-
	L16,L19,L28	-	+	+	-
<i>Streptomyces</i>	L4	-	+	+	-
	L30	-	-	+	-
	R45	+	+	-	+
	R50	-	+	+	+
	R51	+	+	+	+
<i>Streptosporangium</i>	L21	+	+	-	-
Non identified	L22. L25	+	+	+	-
	L5, L38	+	+	+	-

\* L are isolates from leaves and R are isolates from roots.

\*\* + and - = inhibition and absence of inhibition respectively.

The results here described showed that actinomycetes can be isolated as endophytes not only inside maize roots but also inside leaves. The presence of actinomycetes mainly inside root tissues may play an important role in plant development and health. Several reports refer to actinomycetes acting in plant protection against pathogens and the influence of their metabolic products on plant growth and physiology (Katznelson & Cole, 1965 ; Gupta *et al*,1995 and Kolomiets *et al*, 1997). In the present work, the presence of actinomycetes inside roots and leaves of a widely cultivated crop and from a tropical region was confirmed. Certain genera isolated as maize endophytes by us, were also found in the roots of plants from temperate climate (Sardi *et al*, 1992; Matsumoto *et al*, 1998). Other genera as *Streptosporangium* seems to be more frequently found in maize, from a tropical region than in other plants from temperate climate. The results are also as indication of the endophytic diversity that can be found and explored for biotechnological purposes.

## ACKNOWLEDGEMENTS

This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil). We also would like to acknowledge CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for fellowships given to the authors.

## RESUMO

Microrganismos endofíticos são frequentemente encontrados no interior de plantas cultivadas. Embora vários tipos de microrganismos endofíticos tenham sido isolados de milho (*Zea mays*) não existiam dados sobre certos grupos de actinomicetos obtidos a partir desta espécie de planta cultivada. No presente trabalho, actinomicetos endofíticos foram isolados de folhas e raízes de milho. Um total de 53 isolados foram obtidos sendo 31 deles a partir de folhas e 22 a partir de raízes. O gênero *Microbispora* foi o mais

frequente (62%), seguido dos gêneros *Streptomyces* e *Streptosporangium*. Dos actinomicetos isolados, 43,4% apresentaram atividade antimicrobiana contra um ou mais microrganismos ensaiado.

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Received: August 17, 1999;  
Revised: September 15, 1999;  
Accepted: December 27, 1999.