

Isolation and characterization of endophytic bacteria of coffee plants and their potential in caffeine degradation

F. V. Nunes & I. S. de Melo

*Laboratory of Environmental Microbiology,
Embrapa Environment Jaguariúna, SP, Brazil*

Abstract

The plant kingdom is colonized by a diverse array of endophytic bacteria, which form a non-pathogenic relationship with their hosts. Endophytic bacteria may confer benefits to the plant, and the benefits may be reciprocal, resulting in an enhanced symbiotic system for specific plant characteristics. The objective of this study was to isolate endophytic bacteria from coffee (*Coffea arabica* and *C. robusta*) and evaluate their potential in degrading caffeine. The isolates were identified by partial 16S rDNA sequence analysis and by FAME and the analysis of biodegradation of caffeine was carried out by HPLC. As a result, 252 bacterial strains were isolated, with most of them belonging to the species *Bacillus lentimorbus*, *B. megaterium*, *B. subtilis*, *B. cereus*, *Pseudomonas putida*, *P. chlororaphis*, *Pantoea ananatis*, *P. agglomerans*, *Stenotrophomonas malthophilia*, *Kluyvera cryocrescens*, *Kocuria kristinae* etc. Approximately 20% of the bacterial strains showed the ability to grow in the presence of high concentrations (5.000 mg.L⁻¹) of caffeine and two *Pseudomonas putida* strains completely degraded the alkaloid, showing the potential of endophytic bacteria in decaffeination processes.

Keywords: endophytic microorganisms, caffeine, coffee, biodegradation.

1 Introduction

Endophytes are microorganisms that live within the tissues of healthy plants where they cause symptomless infections. Their significance to the host is still



unclear; pathogens as well as non-pathogens can live endophytically. Endophytic bacteria can contribute to the health, growth and development of plants. Plant growth promotion by endophytic bacteria may result from direct effects such as the production of phytohormones or by providing the host plant with fixed nitrogen or the solubilization of soil phosphorus and iron (Glick [1]; Shishido *et al.* [2]; Kinkel *et al.* [3]; Sturz *et al.* [4]). Some endophytic microorganisms have the ability to grow and degrade many xenobiotics. Several studies were carried out to investigate the use of caffeine, as a source of energy for microorganisms. The fungi *Penicillium* and *Aspergillus* and the bacteria *Pseudomonas* are the most frequent caffeine – degrading genera. (Asano *et al.* [5]; Kurtzman and Shwimmer [6]). The objective of this study was to identify endophytic bacteria from coffee and evaluate their potential to degrade caffeine.

2 Material and methods

2.1 Isolation of endophytic bacteria

Leaves, stems and roots of healthy plants of *Coffea arabica* and *C. robusta* were surface sterilized with ethanol 70% per 1 min., sodium hypochlorite 2,5% per 20 min.; and then in ethanol 70% per 30 sec., following three successive washes in distilled sterilized water. The fragments (1 cm) were transferred to tryptic soy-agar medium. The cultures were incubated at 28°C for seven days and the colonies were purified and stored in glycerol at –80°C.

2.2 Strain identification

Isolated strains were screened for the selection of caffeine-resistant strains. Each strain was identified by analysis of fatty acids methyl-esters (FAME) using the Microbial Identification System developed by Microbial ID (MIDI, Neward, DE) and by 16S rDNA sequence analysis.

2.3 Biodegradation of caffeine

The dissipation of caffeine by endophytic bacteria was monitored in mineral medium (Czapeck) supplemented with methylxanthine (5 g.L⁻¹). The analysis of caffeine was carried out on a Shimadzu 10 AS-UP HPLC with photodiode array detector and inertsil 5 ODS-3 (5 µm, 150x4,6 mm) column. The mobile phase was acetic acid/ acetonitrile (90:10, v/v), with a flow rate of 1,0 mL min⁻¹, by an isocratic system.

3 Results and discussion

A total of 252 endophytic bacteria were isolated from coffee, with most of them assigned to the genera *Bacillus*, *Pseudomonas*, *Pantoea*, *Enterobacter*, *Stenotrophomonas*, *Kluyvera* and *Kocuria*. Approximately 20% of the bacterial strains showed the ability to grow in the presence of high concentrations (5.000 mg L⁻¹) of caffeine. Fig. 1 and 2 illustrate the growth of different strains



in Czapeck medium supplemented with 5.000 mg L⁻¹ of 1,3,7-trimethylxanthine. It was observed that two *Pseudomonas putida* strains, 01G and 13R presented the ability to grow in high concentration of caffeine. It is also shown that *Bacillus lentimorbis* (54G), *Enterobacter* sp. (111G), *Alcaligenes xylosoxydans* (06F) and *B. megaterium* (45R and 51R) grew in medium supplemented with caffeine (5.000 µg.mL⁻¹).

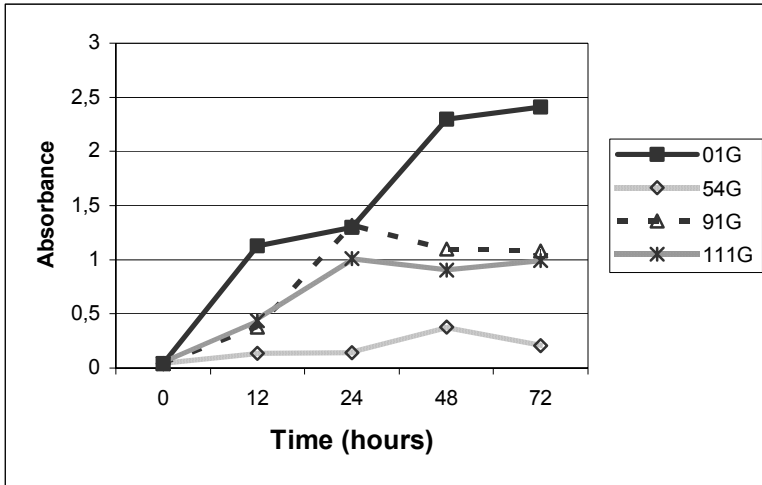


Figure 1: Growth of endophytic bacteria, isolated from coffee stems, supplement with caffeine (5.000 mg L⁻¹): *Pseudomonas putida* (01G), *Bacillus lentimorbis* (54G), *Escherichia coli* (91G) and *Enterobacter* sp. (111G).

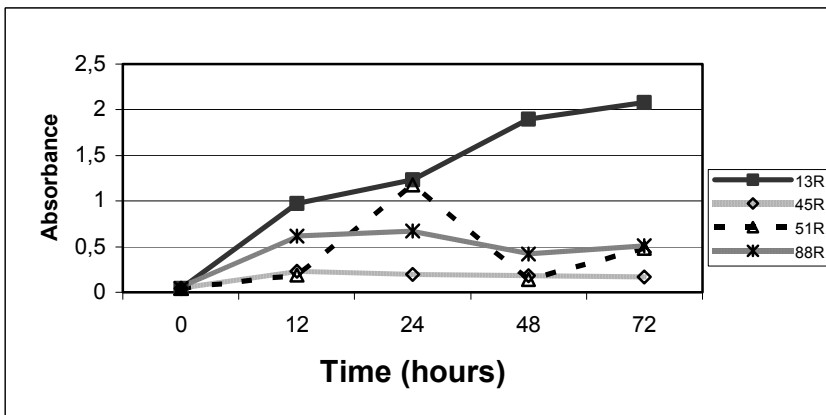


Figure 2: Growth of endophytic bacteria, isolated from coffee roots in liquid medium supplemented with caffeine (5.000 mg L⁻¹): *Pseudomonas putida* (13R), *Bacillus megaterium* (45R), *Bacillus megaterium* (51R) and *Bacillus* sp. (88R).



Twenty endophytic bacterial strains grew in liquid medium containing concentrations as high as 0.05M caffeine. Growth was accompanied by complete removal of caffeine from culture medium. Two *Pseudomonas putida* strains completely degraded the alkaloid (table 1), showing the potential of endophytic bacteria in decaffeination processes. The other bacterial strains evaluated did not metabolized the alkaloid. It is verified that caffeine did not inhibited the normal growth of these bacteria. Caffeine is considered toxic for many microorganisms, however, some microorganisms have the ability to grow in the presence of caffeine and the capacity to degrade this alkaloid.

Table 1: Quantification of caffeine degradation by endophytic bacteria isolated from leaves, stems and roots of *Coffea arabica* and *Coffea robusta*, carried out by HPLC. The initial concentration of caffeine was 5.000 mg L⁻¹.

Treatments	CAFFEINE (g 100 mL ⁻¹)	Treatments	CAFFEINE (g 100 mL ⁻¹)
Control	0,5809	147G- <i>Acinetobacter</i> sp.	0,6571
13R - <i>Pseudomonas putida</i>	0,0000	54G- <i>Bacillus lentimorbus</i>	0,5961
01G - <i>Pseudomonas putida</i>	0,0000	11G-*	0,6076
06F- <i>Alcaligenes xylosoxydans</i>	0,6418	16F- <i>Bacillus cereus</i>	0,6215
12F- <i>Pandoraea</i> sp.	0,6216	43G- <i>Pseudomonas</i> sp.	0,6321
114R- <i>P. putida</i>	0,6278	167G- <i>Stenotrophomonas maltophilia</i>	0,6259
45R- <i>Bacillus megaterium</i>	0,6078	100G- <i>Stenotrophomonas maltophilia</i>	0,6542
7F- <i>Pantoea agglomerans</i>	0,5997	152G-*	0,6168
51R- <i>Bacillus megaterium</i>	0,6370	129G- <i>Ochrobactrum</i> sp.	0,6472
88R- <i>Bacillus</i> sp.	0,6184	91G- <i>Escherichia coli</i>	0,6049
07G- <i>Pseudomonas chlororaphis</i>	0,6357		

*- Bacteria not identified.

One of these strains of *Pseudomonas putida*, strains 13R, presented high pectinolytic activity (pectin lyase) 96,92 nMols of unsaturated product per mL⁻¹. Plant cell walls consist mainly of cellulose, whereas middle lamella, that connects the cells, consists mainly of pectin. Although pectinases might play an important role in plant-microbial interactions and intercellular colonization of roots they have not yet been studied intensively in the endophytic bacteria.



References

- [1] Glick, B.R. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology*, 41: 109-117. (1995).
- [2] Shishido, M., Breuil, C., & Chanway, C.P. Endophytic colonization of spruce by plant growth-promoting rhizobacteria. *FEMS Microbiol. Ecol.* 29:191-196. 1999.
- [3] Kinkel, L.L., Wilson, M., & Lindow, S.E. Plant species and plant incubation conditions influence variability in epiphytic bacterial population size. *Microb Ecol* 39: 1– 11. 2000.
- [4] Sturz, A.V., Christie, B.R. & Nowak, J. Bacterial endophytes: potential role in developing sustainable systems of crop production. *Crit. Rev. Plant Sci.*, v. 19, p. 1-30, 2000.
- [5] Asano, Y., Komeda, T. & Yamada, H. Microbial production of theobromine from caffeine. *Bioscience Biotechnology Biochemistry*, v.57, p.1286-1289, 1993.
- [6] Kurtzman, R.H. & Shwimmer, S. Caffeine removal from growth media by microorganisms. *Experientia*, 127:481-482, 1971.

