

## Title

Endophytic colonization of *Serratia marcescens* in *Medicago sativa* leaves

## Authors

Corral, M.S.<sup>1</sup>, Quelal, M.F.<sup>1</sup>, Salinas, M.T.<sup>1</sup>, De Souza, J.T.<sup>2</sup>, Morales Z.P.<sup>1</sup>

## Institution

<sup>1</sup> Faculty of Engineering and Agricultural Sciences, Universidad de las Américas (UDLA), 170122 Quito, Ecuador, <sup>2</sup>Phytopathology Department, Federal University of Lavras (UFLA), 37200-000 Lavras, MG, Brazil.

## Abstract

Alfalfa, is one of legume species belonging to the family *Fabaceae*. This plant was used in ancient times to feed horses of Central Asia. Now cultivated worldwide, it is a widespread crop in temperate countries. The importance of cultivation alfalfa comes from contribution to animal performance. *Medicago sativa* is an excellent forage plant that provides high levels of protein, minerals and vitamins quality. It is also a source of minerals such as calcium, phosphorus, potassium, magnesium, sulfur, etc. *Serratia marcescens* is a gram-negative bacillus of the family *Enterobacteriaceae*. It is related to growth promotion plants and alterations in the endogenous levels of salicylic acid which gives systemic resistance in cucumber and tobacco. Endophytes have the ability to colonize an ecological niche similar to that of phytopathogens, which makes them suitable biocontrol agents. The aim of this study was test the endophytic colonization of *S. marcescens* rifampicin resistant in *Medicago sativa*. Seeds were surface-sterilized by soaking in 70% ethanol for 1 min, then in 1% sodium hypochlorite for 3 min and rinsed three times with sterile distilled water. Seeds were dried on sterile paper towels and transferred to Petri dishes containing 0.8% water-agar. After incubation at  $25 \pm 2^\circ\text{C}$ , when the radicles measured approximately 1 cm long, germinating seeds were immersed for 30 min in the bacterial suspension containing  $10^5$  CFU ml<sup>-1</sup> of the endophyte. Germinating seeds were transferred to sterile tubes containing 0.8% water-agar and incubated at  $25^\circ\text{C}$  with a 12 h photoperiod. After the incubation, seedlings were removed from the sterile tubes and superficially sterilized as described above for seeds. Roots, stems and leaves were separated, weighed individually, and ground in sterilized mortars with pestles. The resulting extracts were diluted and plated in triplicates onto NA supplemented with  $100 \mu\text{g ml}^{-1}$  of rifampicin. The number of CFU of *S. marcescens* per gram of fresh tissue was determined. Higher levels of *Serratia* colonization occurred in leaves. The results of this study show that bacterial endophytes tend to preferentially colonize certain tissues; this could help to combat pathogens that affect leaf area in alfalfa and others crops.

**Key words:** Endophytic bacteria, Alfalfa, Grazing