The Effect of Temperature Stress on *Rhizobium trifolii* and *Sinorhizobium meliloti* Strains *In Vitro*

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ABSTRACT

Temperature is often pointed out as the major factor in determining the bacterial community diversity and has a marked influence on survival and persistence of rhizobial strains in soils. Rhizobial strains obtained from different red clover and alfalfa varieties were examined for their ability to survive under different temperature levels on two different media. Rhizobial isolates either increased or remained constant as estimated by plate count at 20 and 35°C. Significant reduction was detected at 4, 10, 15 and 42°C in the numbers of CFU for both strains analysed. The differences between the two strains showed that *Sinorhizobium meliloti* survives better than *Rhizobium trifolii* to extreme temperatures.

Keywords: bacterial strains, stress, temperature.

INTRODUCTION

Rhizobia are a genetically diverse and physiologically heterogeneous group of symbiotic nitrogen fixing bacteria that form nodules, within the bacteria fix atmospheric nitrogen into ammonia. Temperature is often pointed out as the major factor in determining the bacterial community diversity, and has a marked influence on survival and persistence of rhizobial strains in soils (Abd-Alla *et al.*, 2014). Temperature affects root hair infection, bacteroid differentiation, nodule structure, and the functioning of the legume root nodule. Many studies have shown that sub-optimal temperatures affect the competitiveness of rhizobia for nodulation, delay root infection and inhibit nodule development and nitrogenase activity. In general, rhizobia have a poor growth at temperatures below 10°C, but they are tolerant to 4°C and for most rhizobia, the optimum temperature range for growth in culture is 28 to 31°C, and many are unable to grow at 37°C (Drouin *et al.*, 2000).

AIMS AND OBJECTIVES

After studying the background of temperature effects on rhizobia we proposed to test different isolates obtained from red clover and alfalfa for their ability to grow at different temperature levels.

MATERIALS AND METHODS

Twenty isolates were identified from colony morphology and gram staining reaction when were cultured on yeast-mannitol agar medium (YEMA). To test the effect of different temperatures (4, 10, 15, 20, 25, 30, 35, 40, 42°C and 28°C as control) on rhizobial strains development it was used a medium with yeast extract, mannitol and soil extract and a medium for aerobic N₂ fixing microorganisms.

RESULTS AND DISCUSSION

Rhizobial isolates cultivated on yeast extract medium showed that the number of CFU was low at 4°C and at 10 and 15°C the differences were

Fig. 1. Rhizobial count CFU/mL $\times 10^7$ at different temperatures on yeast extract medium

Fig. 2. Rhizobial count CFU/mL $\times 10^7$ at different temperatures on a medium for aerobic N_2 fixing microorganisms

insignificant (Fig. 1). On the second medium used the number of CFU either increased or remained constant as estimated by plate count at 25 and 35°C. Significant reduction was detected at 4, 10 and 15°C and 42°C in the numbers of CFU for both strains analysed. And as it was expected at 28°C on both media the number of CFU was higher. On the medium for aerobic N_2 fixing microorganisms the number of colonies was higher for the strains compared to the medium with yeast extract, mannitol and soil extract that could be caused by the complexity of the medium (Fig. 2).

CONCLUSION

Rhizobium strains differ greatly in their sensibility to high and low temperatures. The differences between the two strains showed

that *Sinorhizobium meliloti* survives better than *Rhizobium trifolii* to extreme temperatures.

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