



**NOS-like activity measured in *S.meliloti* IAA-overproducing and control strains.**

### **NOS-like activity assay**

*S. meliloti* cells were grown on minimal medium (10mM phosphate buffer pH 6.3, 7.4 mM sodium succinate, 2.7 mM D-glucose, 0.8 µM nicotinic acid, 1 mM MgSO<sub>4</sub>, 0.5 mM CaCl<sub>2</sub>) with 0.1% NH<sub>4</sub>Cl added as nitrogen source. Cultures were incubated with shaking at 180 rpm at 28°C until reaching stationary phase, harvested by centrifugation at 5000 xg for 20 min and resuspended in Homogenization Buffer (25 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 mM EGTA). Cells were disrupted by sonication for 1 min and the soluble fraction was obtained by centrifugation at 13000 xg for 30 min.

NOS-like activity was determined by the method based on the conversion of L-[<sup>3</sup>H]arginine to L-[<sup>3</sup>H]citrulline, by using the NOS Activity Assay Kit (Cayman Chemical, Ann Arbor, MI). Total protein concentration was measured by Bradford method and the enzyme activity was expressed as fmol arginine µg protein<sup>-1</sup>h<sup>-1</sup>.