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## Antioxidative Enzyme Responses to Antimony Stress of *Serratia marcescens* – an Endophytic Bacteria of *Hedysarum pallidum* Roots

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Table 1. Pearson correlation coefficients between all analyzed variables and between those and antimony culture medium concentrations; \* $p < 0.05$ , \*\* $p < 10^{-2}$ , \*\*\* $p < 10^{-3}$  (n = 15).

	Growth	H <sub>2</sub> O <sub>2</sub>	MDA	Proline	CAT	POD	APX	SOD
Sb (mM)	-0,967***	0,972***	0,108	0,290	0,698**	-0,121	0,422	-0,081
Growth	1	-0,905***	-0,094	-0,233	-0,609*	-0,121	0,422	0,006
H <sub>2</sub> O <sub>2</sub>		1	0,026	0,295	0,727**	-0,267	0,286	-0,247
MDA			1	0,774**	0,536*	0,875***	0,850***	0,773**
Proline				1	0,744**	0,594*	0,760**	0,507*
CAT					1	0,214	0,590*	0,148
POD						1	0,825**	0,959***
APX							1	0,829**

#### MDA Contents

After 24 h of bacterial growth, the MDA contents increased gradually and significantly ( $p < 0.05$ ) at 5 and 10 mM of Sb by 48% and 121%, respectively, compared to the control. At 20 mM of Sb treatment, a non-significant decrease is observed. Then, at 30 mM of Sb the MDA content decreases significantly ( $p < 0.05$ ) with increasing metal stress (Fig. 3c.), and the MDA contents were not correlated with either Sb contents, growth, or H<sub>2</sub>O<sub>2</sub> contents (Table 1). However, when the values obtained at 30 mM of Sb were omitted, the MDA contents become significantly and positively correlated with the Sb ( $r = 0.809$ ,  $p < 10^{-2}$ ,  $n = 12$ ) and H<sub>2</sub>O<sub>2</sub> contents ( $r = 0.587$ ,  $p < 0.05$ ,  $n = 12$ ) and significantly and negatively with growth ( $r = -0.885$ ,  $p < 10^{-2}$ ,  $n = 12$ ).

According to Ayala et al. [33], the lipid peroxidation process produces multiple breakdown molecules, such as MDA, which promotes intramolecular or intermolecular protein/DNA crosslinking, which may induce profound alteration in the biochemical properties of biomolecules. Thus, the significant elevation in the MDA content with the rise of Sb concentrations and the significant and positive correlation of these two parameters of up to 20 mM of Sb, show that the presence of high concentrations of this metalloid in the culture medium can be responsible for the lipid peroxidation of the *S. marcescens* membrane and thus a high MDA production. This one would be responsible for the alteration of the biochemical properties of biomolecules of *S. marcescens*, leading to growth reduction, which is highlighted by the significance and negative correlation of MDA with the growth of the strain up to 20 mM of Sb.

The non-existence of correlation between Sb and MDA contents, when all values are considered, is related to the significant decrease in MDA content at 30 mM of Sb. This can be explained by the fact that a very high amount of H<sub>2</sub>O<sub>2</sub> is produced at 30 mM of Sb, causing an important alteration of the strain metabolic functions, which caused the fall of the MDA production itself. This is also reflected in the lack of correlation between

MDA and H<sub>2</sub>O<sub>2</sub> when all obtained values are introduced. The weakly significant correlation of both variables when obtained values at 30 mM of Sb are omitted can be explained by the fact that other unmeasured ROS than H<sub>2</sub>O<sub>2</sub>, such as OH· and O<sub>2</sub><sup>-</sup>, are involved in the lipid peroxidation process, of which the MDA is the product.

#### Intracellular Proline Contents

The obtained results (Fig. 3d) show that the proline contents of the fresh bacterial biomass increased significantly ( $p < 0.05$ ) with the increase in Sb concentrations. At 5, 10 and 20 mM of Sb, this increase is approximately 59.26%, 65.39% and 114.47% respectively, compared to the control. However, a decrease in the proline content at 30 mM of Sb is noticed with about 39.77% compared to the maximum value. The proline contents were significantly and positively ( $p < 0.05$ ) correlated with MDA contents, CAT, POD, APX, and SOD activities, but they were not correlated with either Sb concentrations, growth, or H<sub>2</sub>O<sub>2</sub> contents (Table 1). However, when we omitted the values obtained at 30 mM of Sb, thus  $n = 12$ , the proline contents became correlated very significantly and positively with the contents of Sb ( $r = 0.907$ ,  $p < 10^{-3}$ ) and H<sub>2</sub>O<sub>2</sub> ( $r = 0.835$ ,  $p < 10^{-2}$ ) and very significantly and negatively to growth ( $r = -0.899$ ,  $p < 10^{-3}$ ).

Proline is an amino acid that acts as a chelating agent for some heavy metals; it reduces the harmful effects of ROS by inhibiting lipid peroxidation because it is a scavenger of the ROS and other free radicals [34]. Thus, its significant increase as a function of Sb concentrations of up to 20 mM of Sb and its positive and significant correlation with MDA and H<sub>2</sub>O<sub>2</sub> contents, show that its production was induced by the presence of elevated concentrations of Sb in order to fight against lipid peroxidation caused by H<sub>2</sub>O<sub>2</sub> toxicity. Furthermore, the negative correlation of proline with growth, for concentrations below 30 mM of Sb, suggests that the reduction of growth, due to Sb toxicity, would be the triggering factor of proline production. Therefore,







of Sb, can be explained, as for APX, by the sensitivity of these enzymes to high H<sub>2</sub>O<sub>2</sub> levels [41]. This explains the significant and positive correlations between these three enzymes and the fact that SOD and POD are only correlated with CAT for values below 10 mM of Sb, which is less sensitive to high H<sub>2</sub>O<sub>2</sub> levels. In the work of Benhamdi et al. [16] the activities of SOD and POD of *H. pallidum* roots were all the higher as soil Sb concentrations were higher. But SOD and POD activities of *S. marcescens* were reduced when Sb concentrations were higher in medium. This could be explained by the fact that, according to Welinder [42], both enzymes differ from those of bacteria.

### Conclusions

*Hedysarum pallidum* Desf., a Fabacea of the semi-arid steppe regions, growing on antimony mining spoils and accumulator of this metalloid, has been found to harbor several endophytic bacteria within its roots. Among these, one strain was able to grow up to 425 mM of Sb in the medium. It was identified as *Serratia marcescens* by sequencing and comparison of its 16S rRNA gene sequence with GenBank data.

The presence of excessive Sb concentrations, corresponding to 3652.8 mg/L, in *S. marcescens* culture medium, caused high accumulation of hydrogen peroxide in the bacteria cells, leading to lipid peroxidation and therefore a decrease in its growth. This latter remained relatively high compared to the control, despite the toxicity of the medium, and reflects an important adaptation of the isolated strain to excessive Sb concentrations. This adaptation also resulted in the ability of the strain to fight ROS by inducing increased production of proline and antioxidant enzymes, such as CAT and APX, at the most excessive Sb doses. The induction of SOD and POD activities, very sensitive enzymes to H<sub>2</sub>O<sub>2</sub> excess, up to Sb concentrations reaching levels of 1216.6 mg/L, is another proof of the adaptation of *S. marcescens* to Sb toxicity. Correlations show that the strain implements, at the same time, its antioxidant molecules to fight the antimony stress up to the threshold of 1216.6 mg/L Sb, but when this threshold is exceeded only the least sensitive molecules are involved, namely proline, CAT and APX.

The strain's ability to produce a sufficient biomass up to 3652.8 mg/L Sb in the culture medium and to produce high levels of antioxidant biomarkers attests of its high resistance to excessive levels of antimony. Such an ability, similar to that of its host, suggests that *S. marcescens* is involved in the aptitude of its host, *Hedysarum pallidum*, to grow on the mining spoils and to accumulate antimony. This could make *Serratia marcescens*, alone or associated with its plant host, a potential candidate for bioremediation of antimony-contaminated soils.

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### Conflict of Interest

The authors declare no conflict of interest.

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