

Prospection of Resistant Antibacterial Peptides from Soybean [*Glycine max* L. (Merrill)] by using Peptidomic Tools

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The peptides are widely distributed in living systems and participate in key regulatory and defense processes. Considering the time of the outbreak of seeds as a stress situation for the tissues, resistant AMPS were prospected in soybean seeds germinated for 48 hours (SSG48). This work aimed to establish a procedure to localize and identify antimicrobial peptides (AMPs) with resistance to adverse conditions, for biotechnological application. Different techniques for clearance and purification of the extracts were tried. SSG48 were triturated, macerated (Tris containing protease inhibitors), followed by salt fractionation, selective heating, and molecular exclusion (MEC) and reversed phase (RP) chromatography in HPLC. SDS-Tricine-PAGE of samples after MEC and heating of the recovered fractions suggested aggregation of peptides. Best fractionation and enrichment of peptide were obtained by MEC in the presence of 0.2 M NaCl and RP-HPLC. The fractions obtained after RP-HPLC were heated (boiling for 2h) and used for evaluation of the antibacterial activity and mass spectrometry analysis. Growth inhibition values up to 83% and 98% were observed for the plant-pathogens *Ralstonia solanacearum* and *Clavibacter michiganensis* ssp. *michiganensis*, respectively. Molecular masses (2-9 kDa) were identified in these fractions. The established protocol allowed the enrichment of resistant AMPs in the SSG48, being suitable for evaluation of the peptidomic of seed germination. Furthermore, it was possible to detect the presence of several peptide fractions with antibacterial activity that are resistant to adverse conditions, appropriated to be exploited for biotechnological application in agribusiness.

Keywords: Resistant Antibacterial Peptides, Peptidomic Tools, Soybean, Seeds germinated

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