

# EVALUATION OF THE POTENTIAL APPLICATION OF HYICIN 3682, A STAPHYLOCOCCIN WHICH INHIBITS THE GROWTH OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* IN TOMATO SEEDS

**Authors** Fagundes, P.C., Kornetz, J.A.S.P., Romanos, M.T.V., Bastos, M.C.F.

**Institution** Instituto de Microbiologia Paulo de Góes - Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho, 373 – CCS - Bloco I - Cidade Universitária, 21941-902 - Rio de Janeiro – RJ - Brasil)

## Abstract

Hyicin 3682 is a lantibiotic produced by *Staphylococcus hyicus* 3682, a strain isolated from bovine milk. The main goals of the present study were to determine the spectrum of activity and to investigate the potential biotechnological applications of hyicin 3682. This bacteriocin exhibited a broad spectrum of inhibitory activity against many Gram-positive bacteria, including food pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*, and the tomato phytopathogen *Clavibacter michiganensis* subsp. *michiganensis*, which causes the bacterial canker. No toxic effect to eukaryotic cell types L-929 and Raw was observed and the  $CC_{50}$  was 1.614 and  $>1.686$  mg/ml, respectively. A partially-purified hyicin 3682 preparation obtained after cation-exchange chromatography (10,240 AU/ml) revealed no hemolytic activity against sheep erythrocytes. Hyicin 3682 was sensitive to both the action of simulated gastric juice and bile salts. The half-life of hyicin 3682 (51,200 AU/ml) was determined during storage at temperatures of 25, 4 and  $-20^{\circ}\text{C}$  for 52 weeks. The results indicated that this bacteriocin is quite stable for 8 weeks at 25 and  $4^{\circ}\text{C}$ , and for 12 weeks at  $-20^{\circ}\text{C}$ . A residual activity, which varied from 800 to 12,800 AU/ml, was observed at end of the experiment. Hyicin 3682 proved to be bactericidal, causing simultaneous lysis, against *Micrococcus luteus* ATCC 4698 and *C. michiganensis* subsp. *michiganensis* Cmm4. The lysis against *S. aureus* 4S1, a strain isolated from salad, occurred less intensely and belatedly. An alginate/gelatin matrix, containing 192 AU of hyicin 3682, was able to inhibit the growth of the phytopathogen *C. michiganensis* subsp. *michiganensis* Cmm4, giving rise to inhibition zones of  $5.1 \pm 0.2$  cm<sup>2</sup>. Inhibition zones of  $3.2 \pm 0.2$  cm<sup>2</sup> against *M. luteus* ATCC 4698 and of  $3.7 \pm 0.4$  cm<sup>2</sup> against *C. michiganensis* subsp. *michiganensis* Cmm4 were also observed after adsorption of hyicin 3682 to sections of a polyethylene plastic used for food packaging. Experiments with tomato seeds (*Solanum lycopersicum*) revealed that hyicin 3682 (3,000 AU) seems not to interfere with the plant development and to promote the inhibition of *C. michiganensis* subsp. *michiganensis* Cmm4, precluding the development of plants with symptoms of the bacterial canker. Therefore, hyicin 3682 has potential applications as a biopreservative for food and as a possible alternative to control the *C. michiganensis* subsp. *michiganensis* growth in tomato crops.

**Key words:** hyicin 3682, lantibiotic, *Clavibacter michiganensis* subsp. *michiganensis*, tomato

**Funding Agencies:** CNPq, CAPES and FAPERJ