Insecticidal Proteins Produced by *Enterococcus faecalis* Isolated from Great Wax Moth, *Galleria mellonella*

Shin Yong Park, Sang Hak Lee, Kyung Yeon Cho, Chang Soo Kang and In Hee Lee

Department of Life Science, Hoseo University

Since the availability of orally active insecticidal proteins outside the Bt endotoxin family has been limited to date, several toxins secreted from a variety of entomopathogenic bacteria have attracted considerable interests in the hope of developing new types of biological insecticides. We have recently isolated a novel entomopathogenic bacterium, *Enterococcus faecalis* (Gm), from the hemolymph of *G. mellonella* larvae. Although there has been no report about the finding or the possible activity in which *Enterococcus* spp. is virulent for insects. Our *E. faecalis* (Gm) from *G. mellonella* showed a prominent toxicity against host insects. The present study has focused on finding of proteins produced by *E. faecalis*, which might contribute to the insecticidal activities. First, we compared proteins in secretions from *E. faecalis* (Gm) with those of other three *E. faecalis* strains (ATCC), confirmed to be lacking insecticidal activities, on SDS-PAGE gel. *E. faecalis* (Gm) secreted at least two distinct proteins (P28, 28 kDa; P43, 43 kDa), which were not detected in the culture media of three *E. faecalis* ATCC strains. We have isolated and purified two potential toxins from a culture broth for *E. faecalis* (Gm) by a consecutive four-step procedures consisting of ultrafiltration, gel permeation chromatography, anion-exchange HPLC and reverse-phase HPLC. In the bioassay performed with the purified proteins, they retain a decreased insecticidal efficiency to *G. mellonella* larvae. Of two purified proteins, P28 was N-terminally sequenced by Edman degradation. Our database search revealed that P28 is highly homologous to serine protease found in *E. faecalis*. Further experiments including gene cloning and protease assay for P28 generated data to support the fact that it is a serine protease.