

Three Fungus-Binding Proteins in the Hemolymph of *Galleria mellonella* Larvae

**Eun Kyung Yun, Kyu Nam Kim, Joon Ha Lee, Young Shin Lee,
Ik Soo Kim¹ and In Hee Lee**

Department of Life Science, Hoseo University,

¹Department of Sericulture and Entomology,

National Institute of Agriculture Science and Technology

We used a heat-killed fungus, *Candida albicans*, to identify fungi-binding proteins in the hemolymph of the naive or the immunized *Galleria mellonella* larvae with *E. coli*. After *C. albicans* ($> 2 \times 10^7$ cells) were incubated with hemolymph at room temperature. Proteins-bound *C. albicans* were separated by centrifugation and washed two times. Binding proteins were detached from fungi in an acidic buffer containing 0.5 M NaCl and they were directly subjected to C18 reverse-phase HPLC column. In the profile of HPLC performed with sample of immunized hemolymph, we confirmed at least three prominent peaks (peaks 1, 2 and 3). Of three peaks, peak 1 was also identified in the sample of normal hemolymph. In contrast, other two peaks were detected only in the immunized hemolymph. Proteins in each peak were monitored on SDS-PAGE gel and N-terminally sequenced by Edman degradation after transferring protein band to PVDF membrane. Database searches on amino acid sequences of proteins in each peak revealed that peak 1 contains a serine protease inhibitor and peak 2 includes lysozymes. Peak 3 had two proteins, which have not been sequenced yet.

We surmise that fungi-binding proteins might play a critical role to remove (or kill) invading fungi into the insect hemocoel. However we could not detected anti-candidacidal activities of proteins of three peaks in to date experiments. If the function of fungi-binding proteins are explained in our present work, it might be a key to solve an enigma as to the defense strategy of insects against invading fungi.