Part III: Antibacterial Protein in Serum Hemolymph and Hemocyte Lysate Supernatant of *Penaeus merguiensis*

1. *Penaeus merguiensis* Hemocytes

In *P. merguiensis*, there are three types of circulating cells under a light microscope. The one type of hyaline cell observed in shrimp hemolymph was considered as a typical oval or elonged shape, agranule and measured approximately 2.8 μm. Two subgroups of granulocytes, small granule hemocyte (SGH) has rounded shape, 2.5 μm and small intracellular granules. The second type of the cells, large granule hemocyte (LGH) was detected with 2 μm and contain numerous dark large granules as shown in Figure 26. According to the type of hemocyte, the total hemocyte count (THC) and different cell counts were measured by light microscope. Mean THC was $4.74\pm1.32x10^{6}$ cells/ml. The viable cell populations withdrawn from the banana prawn consisted of 56±15% viable cells. The percentage of differential hemocyte counts are shown in Figure 27. Small granule hemocyte was the most abundant population, 83.3±9.4% and the LGH was 8.2±4% while hyaline was the least abundant 5±2%.

The SDS-PAGE of hemolymph serum and hemocyte lysate supernatant (HLS) proteins performed by using silver staining showed that the protein from hemolymph and serum could be divided into fifteen clear bands while the protein from HLS with three or four bands which three bands were markedly dominants of Mr 69.5, 57.0 and 43.5 as shown in Figure 28. The band of Mr 57.0 kDa was detected in hemolymph serum and HLS.

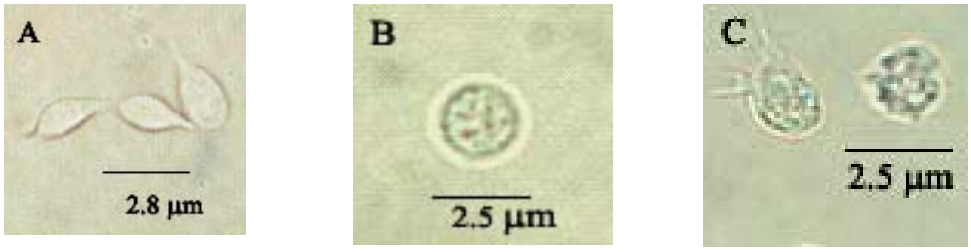

Figure 26 Light micrographs of shrimp hemocytes, *Penaeus merguiensis*. (A) hyaline hemocytes; (B) a small granule hemocyte (SGH); (C) large granule hemocytes (LGH).

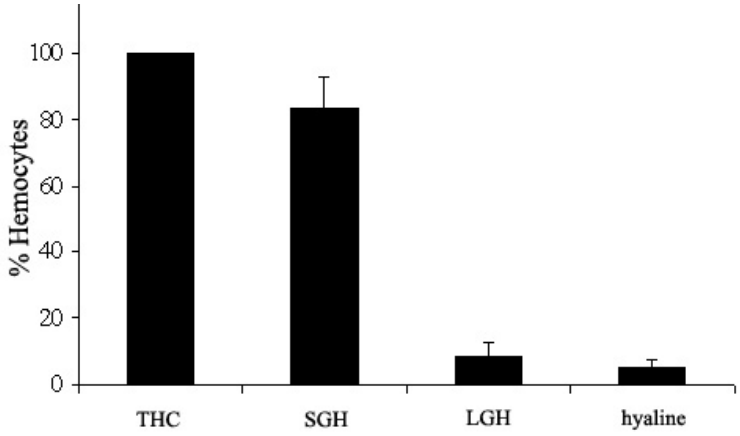

Figure 27 Total and differential counts of hemocytes in *Penaeus merguiensis* hemolymph (values are averge±SD, n=30) Total hemocyte count (THC), small granule hemocyte (SGH), large granule hemocyte (LGH)

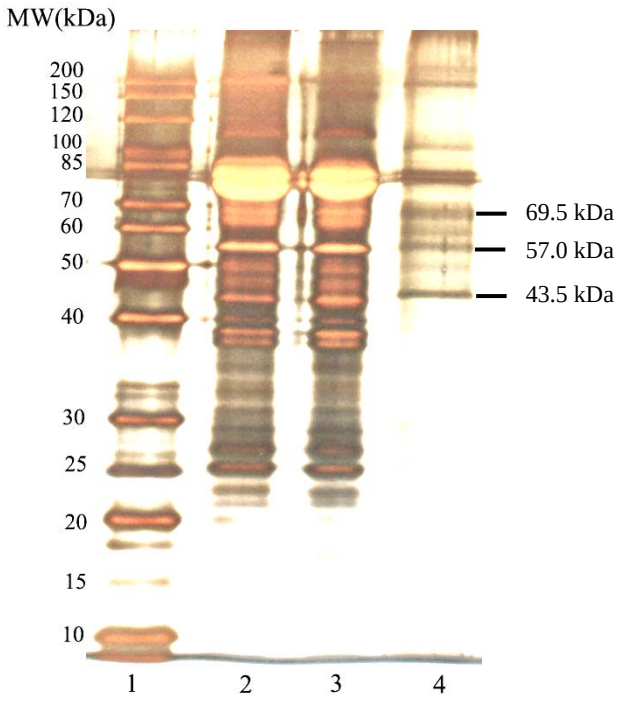

Figure 28 12.5% SDS-PAGE of hemolymp (lane 2) serum (lane 3) and hemocyte lysate supernatant (lane 4) proteins in reducing condition. Lane 1: molecular weight marker proteins. The proteins in lanes 2 and 3 were 2 µg proteins while lane 4 was 5 µg proteins. The protein in samples were treated with dithiothreitol (DTT). Protein bands were visualized with Silver staining.

2. Hemagglutinin Activity

The agglutination by the serum hemolymph and HLS of *P. merguiensis* using human and animal erythrocytes as agglutinnogens is shown in Table 13. All test samples caused agglutinating activity with at least one type of erythrocytes. Agglutination of rabbit erythrocyte was higher than that of the other mammalian species tested, and their agglutination titers increased when trypsinised human A cells were used. The maximum titers were produced by serum and hemolymph against

trypsin treated human A erythrocytes while HLS agglutinated rabbit erythrocytes. Hence, the serum hemolymph and HLS may contained more than one type of lectins. HLS may gave difference lectins from serum and hemolymph.

Table 13 Hemagglutinating activity (HA) of serum, hemolymph and haemocyte lysate supernatant from *P. merguiensis* against different types of mammalian and animal erythrocytes.

Erythrocytes	HA (titer)		
	serum	hemolymph	Hemocyte lysate supernatant
Human A	64	16	0
Trypsin treated Human A	256	256	16
Human B	32	16	0
Human O	32	16	0
Sheep	128	0	0
Rabbit	128	64	256
Guinea pig	64	0	0
Rat	64	0	0
Hamster	0	0	0
Protein (mg/ml)	143.0	30.50	0.1411 (n = 9)

3. Antibacterial activity

The antibacterial activity of serum, affinity purified lectin, gel filtration purified lectin (~ 1.28 μg proteins) and HLS (~ 2 μg proteins) from hemolymph of banana prawn is present in Figure 29. The serum, HLS and affinity purified lectin exhibited approximately 20% antibacterial effect against all test strains of *Vibrio* species. According to affinity purified lectin had strong inhibitory effect on *V. angillarum*, *V. cholerae* and *V. fluvialis* with 68.75, 62.05 and 61.16%, respectively. Serum of the banana prawn demonstrated antibacterial activity against *Vibrio* species ranging from 17.64-45.32%, but no inhibitory effect with *V. harveyi*. Serum of the banana prawn showed the highest antibacterial activity against *V. alginolyticus* approximately 45%. HLS extracted from the hemocyes of banana prawn contained antibacterial activity. HLS had slight inhibitory against all test strain bacteria which affect *Vibrio* species ranged from 24.94-57.83% and gave the highest inhibitory against *V. parahemolyticus*. Using 2 μg proteins showed antibacterial activity against *E.coli* and *S. aureus* with 56.85 and 29.98%, respectively. In liquid growth inhibition of each assays, HLS, affinity purified lectin and gel filtration purified lectin had highly inhibitory marked activity against both gram-negative and gram-positive bacteria. Nevertheless, HLS, affinity purified lectin and gel filtration purified lectin exhibited more antibacterial activity on gram-negative than gram-positive bacteria, *S. aureus*. Among the gram-negative bacteria, *V. alginolyticus*, *V. cholerae* and *V. harveyi* affinity purified lectin was two times more active than HLS at 1.28 and 2 μg proteins. Apparently, gel filtration purified lectin gave higher activity than affinity purified lectin. However, no activity was found for serum against *V. harveyi* and *S. aureus*.

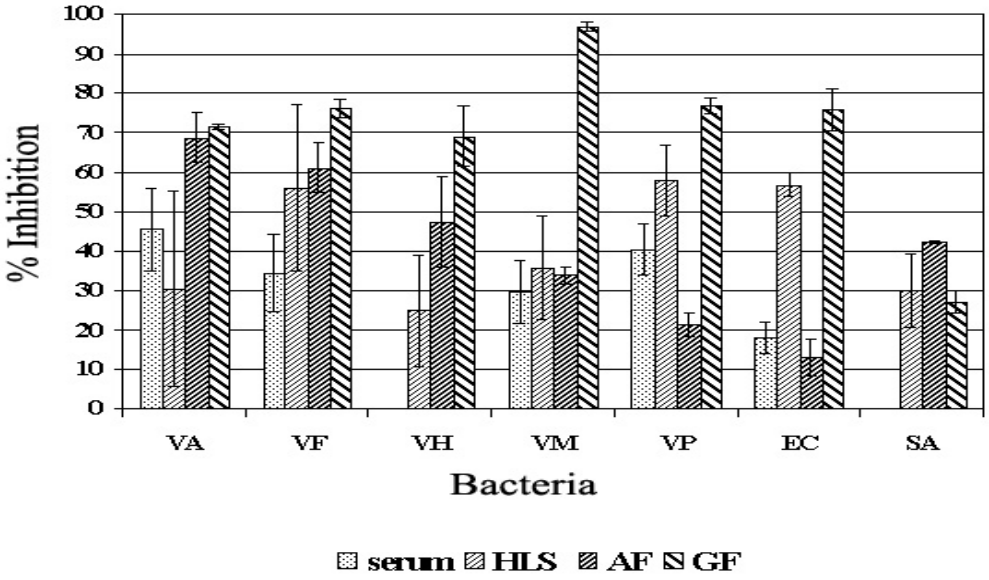

Figure 29 Antibacterial activity of serum, affinity purified lectin (AF) and gel filtration purified lectin (GF) (~ 1.28 μg proteins) and HLS (~ 2 μg proteins) from hemolymph and hemocyte of *Penaeus merguiensis*. **(**VA = *V. alginolyticus*, VF = *Vibrio fluvialis*, VH = *V. harveyi*, VM = *V. mimicus*, VP = *V. parahemolyticus*, EC = *Escherichia coli* and SA = *Staphylococcus aureus*)