Functional characterization of phagocytes in the Pacific oyster *Crassostrea gigas*

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**RAW DATA**

Fig. 1. (B) The percentages of haemocyte subpopulations were calculated by flow cytometry.

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| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| agranulocytes (%) | 45.1 | 43.0 | 49.3 | 53.1 | 45.7 | 40.8 |
| granulocytes (%) | 35.7 | 28.5 | 26.9 | 32.3 | 33.4 | 31.5 |
| semigranulocytes (%) | 19.1 | 22.3 | 18.6 | 20.5 | 18.7 | 18.5 |

Fig. 3. The phagocytic percentages of total haemocytes, granulocytes and semigranulocytes were calculated by flow cytometry. TH, G and SG are short for total haemocytes, granulocytes and semigranulocytes respectively, RGD is short for Arg-Gly-Asp tripeptide.

(B) The phagocytic percentages of haemocytes towards *E. coli*.

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| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| TH (%) | 24.5 | 26.7 | 11.3 | 36.1 | 24.9 | 25.1 |
| TH + RGD (%) | 8.4 | 13.6 | 10.2 | 14.5 | 14.1 | 23.5 |
| G (%) | 21.3 | 35.7 | 36.3 | 40.6 | 39.5 | 58.6 |
| G + RGD (%) | 23.9 | 12.1 | 25.5 | 24.7 | 33.8 | 24.4 |
| SG (%) | 18.0 | 18.3 | 29.5 | 19.8 | 18.9 | 10.3 |
| SG + RGD (%) | 20.3 | 10.1 | 4.5 | 6.2 | 10.5 | 9.8 |

(D) The phagocytic percentages of haemocytes towards *V. splendidus*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| TH (%) | 10.8 | 8.6 | 8.9 | 8.1 | 8.5 | 4.5 |
| TH + RGD (%) | 4.1 | 4.2 | 5.4 | 5.3 | 5.9 | 4.8 |
| G (%) | 14.4 | 10.9 | 7.8 | 6.9 | 9.5 | 9.1 |
| G + RGD (%) | 5.8 | 8.4 | 6.2 | 6.7 | 5.1 | 5.9 |
| SG (%) | 5.5 | 6.9 | 6.2 | 8.1 | 7.1 | 10.8 |
| SG + RGD (%) | 3.6 | 4.1 | 4.3 | 3.6 | 3.2 | 4.3 |

(F) The phagocytic percentages of haemocytes towards *S. aureus*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| TH (%) | 26.1 | 13.9 | 15.8 | 10.1 | 13.4 | 9.1 |
| TH + RGD (%) | 6.1 | 7.4 | 2.7 | 7.6 | 13.2 | 11.0 |
| G (%) | 36.7 | 24.1 | 23.9 | 14.6 | 20.7 | 24.3 |
| G + RGD (%) | 20.2 | 14.4 | 13.7 | 13.1 | 14.2 | 10.1 |
| SG (%) | 10.9 | 9.6 | 5.5 | 10.1 | 16.1 | 9.8 |
| SG + RGD (%) | 3.1 | 5.4 | 4.9 | 9.9 | 4.8 | 3.5 |

Fig. 4. The relative phagocytic activities of total haemocytes, granulocytes and semigranulocytes after carbohydrate incubation were calculated by flow cytometry. TH, G and SG are short for total haemocytes, granulocytes and semigranulocytes respectively, Glu, Fuc, Man, Lac and GlcNAc are short for glucose, fucose, mannose, lactose and N-acetylglucosamine respectively.

(A) The relative phagocytic activities of total haemocytes towards *V. splendidus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| TH (%) | 103.4 | 93.7 | 97.2 | 93.6 | 110.1 | 103.2 |
| TH + Glu (%) | 95.6 | 90.1 | 84.8 | 96 | 104.7 | 101.8 |
| TH + Fuc (%) | 84.2 | 61.6 | 33.6 | 22.5 | 51.4 | 97.1 |
| TH + Man (%) | 98.2 | 85.5 | 54.4 | 81.8 | 31.3 | 51.2 |
| TH + Lac (%) | 41.3 | 79.2 | 59.4 | 64.7 | 94.1 | 92.6 |
| TH + GlcNAc (%) | 40.5 | 42.9 | 92.2 | 66.5 | 96.3 | 49.4 |

(B) The relative phagocytic activities of granulocytes towards *V. splendidus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| G (%) | 126.2 | 96.7 | 78.3 | 115.4 | 87.5 | 98.1 |
| G + Glu (%) | 113.5 | 97.6 | 75.7 | 98.3 | 106.6 | 82.4 |
| G + Fuc (%) | 70.5 | 48.3 | 98.9 | 69.7 | 76.1 | 77.2 |
| G + Man (%) | 77.2 | 88.1 | 93.6 | 90.4 | 69.8 | 65.6 |
| G + Lac (%) | 74.9 | 66.6 | 71.7 | 70.4 | 86.2 | 83.4 |
| G + GlcNAc (%) | 74.8 | 98.3 | 64.8 | 49.1 | 72.9 | 73.4 |

(C) The relative phagocytic activities of semigranulocytes towards *V. splendidus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| SG (%) | 82.9 | 80.6 | 103.5 | 118.4 | 105.6 | 108.5 |
| SG + Glu (%) | 118.3 | 95.4 | 93.2 | 108.6 | 96.7 | 64.8 |
| SG + Fuc (%) | 88.6 | 76.5 | 19.7 | 86.3 | 27.8 | 29.8 |
| SG + Man (%) | 47.4 | 19.2 | 46.9 | 88.7 | 26.1 | 86.3 |
| SG + Lac (%) | 90.3 | 73.6 | 37.8 | 88.4 | 27.7 | 47.8 |
| SG + GlcNAc (%) | 81.7 | 78.3 | 27.9 | 72.6 | 26.9 | 86.4 |

(D) The relative phagocytic activities of total haemocytes towards *S. aureus.*

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| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| TH (%) | 66.7 | 98.3 | 97.1 | 118.4 | 106.4 | 115.6 |
| TH + Glu (%) | 93.7 | 77.6 | 98.4 | 107.4 | 94.8 | 106.0 |
| TH + Fuc (%) | 43.2 | 65.3 | 64.8 | 99.2 | 82.5 | 69.7 |
| TH + Man (%) | 26.9 | 81.6 | 78.2 | 84.3 | 58.3 | 34.1 |
| TH + Lac (%) | 80.3 | 99.8 | 96.7 | 104.5 | 73.6 | 107.2 |
| TH + GlcNAc (%) | 70.6 | 81.2 | 62.9 | 50.4 | 29.7 | 88.1 |

(E) The relative phagocytic activities of granulocytes towards *S. aureus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| G (%) | 128.5 | 105.2 | 86.4 | 62.6 | 107.5 | 112.1 |
| G + Glu (%) | 117.8 | 66.4 | 109.2 | 78.7 | 107.5 | 84.3 |
| G + Fuc (%) | 60.7 | 27.4 | 63.5 | 69.8 | 85.2 | 71.3 |
| G + Man (%) | 78.7 | 66.4 | 33.5 | 20.7 | 80.6 | 69.2 |
| G + Lac (%) | 62.4 | 96.5 | 117.3 | 90.8 | 106.6 | 91.7 |
| G + GlcNAc (%) | 54.5 | 21.8 | 95.7 | 32.8 | 58.6 | 75.3 |

(F) The relative phagocytic activities of semigranulocytes towards *S. aureus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| SG (%) | 85.3 | 116.8 | 119.5 | 101.4 | 67.6 | 108.7 |
| SG + Glu (%) | 69.3 | 111.6 | 107.5 | 74.8 | 93.3 | 104.2 |
| SG + Fuc (%) | 93.6 | 45.9 | 78.7 | 80.5 | 52.6 | 85.3 |
| SG + Man (%) | 59.3 | 88.9 | 64.5 | 42.7 | 75.6 | 90.1 |
| SG + Lac (%) | 108.4 | 96.9 | 107.5 | 94.3 | 78.7 | 71.6 |
| SG + GlcNAc (%) | 83.6 | 86.3 | 69.1 | 75.6 | 81.5 | 87.0 |

Fig. 5. The relative phagocytic activities of total haemocytes, granulocytes and semigranulocytes after LPS and PGN incubation were calculated by flow cytometry respectively. TH, G and SG are short for total haemocytes, granulocytes and semigranulocytes respectively, LPS and PGN are short for lipopolysaccharide and peptidylglycan respectively.

(A) The relative phagocytic activities of total haemocytes towards *V. splendidus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| TH (%) | 113.6 | 105.7 | 102.6 | 88.4 | 86.7 | 102.8 |
| TH + 0.01 mg/ml LPS (%) | 142.6 | 106.9 | 103.4 | 110.1 | 128.5 | 115.5 |
| TH + 0.1 mg/ml LPS (%) | 133.8 | 179.7 | 88.1 | 132.7 | 164.5 | 167.2 |
| TH + 0.01 mg/ml PGN (%) | 113.5 | 96.7 | 72.6 | 118.4 | 65.4 | 107.2 |
| TH + 0.1 mg/ml PGN (%) | 74.8 | 108.1 | 95.7 | 86.8 | 97.4 | 115.6 |

(B) The relative phagocytic activities of granulocytes towards *V. splendidus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| G (%) | 76.7 | 98.9 | 105.4 | 97.5 | 121.4 | 100.7 |
| G + 0.01 mg/ml LPS (%) | 110.8 | 108.9 | 125.2 | 113.7 | 111.6 | 99.1 |
| G + 0.1 mg/ml LPS (%) | 123.3 | 118.5 | 116.4 | 141.6 | 96.9 | 115.8 |
| G + 0.01 mg/ml PGN (%) | 118.2 | 95.4 | 77.5 | 81.8 | 97.2 | 105.6 |
| G + 0.1 mg/ml PGN (%) | 113.9 | 75.4 | 97.1 | 93.5 | 107.4 | 83.8 |

(C) The relative phagocytic activities of semigranulocytes towards *V. splendidus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| SG (%) | 102.8 | 85.9 | 71.7 | 98.6 | 132.2 | 110.6 |
| SG + 0.01 mg/ml LPS (%) | 134.2 | 151.8 | 122.6 | 96.9 | 113.7 | 133.8 |
| SG + 0.1 mg/ml LPS (%) | 117.8 | 172.1 | 139.7 | 163.8 | 140.4 | 187.9 |
| SG + 0.01 mg/ml PGN (%) | 112.3 | 98.6 | 84.5 | 73.8 | 91.1 | 105.2 |
| SG + 0.1 mg/ml PGN (%) | 72.7 | 108.9 | 93.4 | 85.2 | 118.4 | 96.1 |

(D) The relative phagocytic activities of total haemocytes towards *S. aureus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| TH (%) | 115.7 | 113.5 | 102.7 | 83.6 | 98.2 | 87.6 |
| TH + 0.01 mg/ml LPS (%) | 131.1 | 109.3 | 114.9 | 118.6 | 103.2 | 123.7 |
| TH + 0.1 mg/ml LPS (%) | 145.7 | 99.3 | 134.8 | 141.2 | 106.7 | 161.8 |
| TH + 0.01 mg/ml PGN (%) | 94.7 | 78.6 | 117.2 | 91.8 | 82.6 | 105.4 |
| TH + 0.1 mg/ml PGN (%) | 97.8 | 105.4 | 87.1 | 76.9 | 118.6 | 90.4 |

(E) The relative phagocytic activities of granulocytes towards *S. aureus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| G (%) | 108.2 | 87.5 | 93.6 | 105.4 | 112.7 | 94.8 |
| G + 0.01 mg/ml LPS (%) | 125.4 | 104.8 | 115.4 | 119.5 | 114.2 | 106.3 |
| G + 0.1 mg/ml LPS (%) | 154.7 | 159.5 | 119.2 | 117.5 | 108.4 | 117.1 |
| G + 0.01 mg/ml PGN (%) | 79.2 | 107.5 | 96.8 | 90.5 | 102.8 | 87.1 |
| G + 0.1 mg/ml PGN (%) | 98.4 | 80.8 | 107.6 | 95.9 | 76.2 | 106.7 |

(F) The relative phagocytic activities of semigranulocytes towards *S. aureus.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Haemocytes | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
| SG (%) | 98.6 | 116.3 | 104.2 | 95.8 | 82.1 | 102.6 |
| SG + 0.01 mg/ml LPS (%) | 134.7 | 120.9 | 128.6 | 117.4 | 118.5 | 96.8 |
| SG + 0.1 mg/ml LPS (%) | 126.1 | 109.6 | 162.9 | 166.5 | 131.2 | 108.7 |
| SG + 0.01 mg/ml PGN (%) | 92.7 | 87.6 | 108.4 | 72.6 | 106.8 | 104.4 |
| SG + 0.1 mg/ml PGN (%) | 90.8 | 108.6 | 97.2 | 89.8 | 103.2 | 95.4 |

Fig. 6. (B) The percentages of haemocytes gated on PE+/FITC+, PE+/FITC- and PE-/FITC+ were calculated by flow cytometry.

Wheat germ agglutinin staining

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Microbes | Fluorescence | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
| *E. coli* | PE+/FITC+ (%) | 20.4 | 19.7 | 31.8 | 24.6 | 12.5 |
| PE+/FITC- (%) | 16.4 | 28.9 | 25.7 | 30.6 | 24.6 |
| PE-/FITC+ (%) | 9.7 | 17.2 | 14.8 | 11.7 | 12.6 |
| *V. splendidus* | PE+/FITC+ (%) | 17.9 | 9.8 | 12.7 | 16.4 | 10.2 |
| PE+/FITC- (%) | 29.6 | 24.2 | 33.6 | 39.7 | 42.3 |
| PE-/FITC+ (%) | 18.9 | 12.5 | 17.4 | 21.8 | 18.1 |
| *B. subtilis* | PE+/FITC+ (%) | 25.8 | 12.7 | 15.9 | 20.6 | 19.4 |
| PE+/FITC- (%) | 47.8 | 40.1 | 29.6 | 36.7 | 33.9 |
| PE-/FITC+ (%) | 10.3 | 9.7 | 18.4 | 13.6 | 15.8 |
| *S. aureus* | PE+/FITC+ (%) | 33.2 | 27.6 | 23.4 | 39.8 | 30.5 |
| PE+/FITC- (%) | 30.6 | 38.6 | 32.7 | 27.6 | 42.1 |
| PE-/FITC+ (%) | 7.5 | 12.6 | 8.6 | 10.5 | 8.7 |

Peanut agglutinin staining

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Microbes | Fluorescence | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
| *E. coli* | PE+/FITC+ (%) | 15.7 | 7.9 | 9.2 | 7.6 | 10.4 |
| PE+/FITC- (%) | 12.7 | 18.9 | 16.2 | 17.4 | 10.8 |
| PE-/FITC+ (%) | 15.4 | 20.7 | 15.1 | 19.8 | 17.9 |
| *V. splendidus* | PE+/FITC+ (%) | 3.8 | 7.9 | 9.4 | 5.7 | 4.1 |
| PE+/FITC- (%) | 20.6 | 16.8 | 14.7 | 17.5 | 15.2 |
| PE-/FITC+ (%) | 7.5 | 15.4 | 9.2 | 12.3 | 11.8 |
| *B. subtilis* | PE+/FITC+ (%) | 5.8 | 9.2 | 8.4 | 7.8 | 7.1 |
| PE+/FITC- (%) | 7.8 | 15.6 | 11.2 | 13.6 | 8.9 |
| PE-/FITC+ (%) | 18.9 | 12.2 | 16.7 | 19.5 | 14.8 |
| *S. aureus* | PE+/FITC+ (%) | 9.5 | 8.6 | 5.2 | 7.8 | 6.4 |
| PE+/FITC- (%) | 19.3 | 16.7 | 12.1 | 15.4 | 11.9 |
| PE-/FITC+ (%) | 15.4 | 18.8 | 19.2 | 11.7 | 17.6 |

*Lycopersicon esculentum* lectin staining

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Microbes | Fluorescence | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
| *E. coli* | PE+/FITC+ (%) | 24.9 | 24.8 | 19.4 | 21.6 | 25.5 |
| PE+/FITC- (%) | 3.9 | 4.3 | 2.5 | 3.1 | 2.2 |
| PE-/FITC+ (%) | 3.6 | 2.7 | 1.9 | 1.5 | 2.4 |
| *V. splendidus* | PE+/FITC+ (%) | 25.6 | 20.1 | 15.1 | 17.6 | 15.3 |
| PE+/FITC- (%) | 2.7 | 5.6 | 4.3 | 3.8 | 4.2 |
| PE-/FITC+ (%) | 1.8 | 2.6 | 1.4 | 2.3 | 2.7 |
| *B. subtilis* | PE+/FITC+ (%) | 22.4 | 21.6 | 29.5 | 27.6 | 19.7 |
| PE+/FITC- (%) | 1.9 | 3.8 | 2.1 | 2.9 | 3.0 |
| PE-/FITC+ (%) | 0.9 | 2.8 | 1.6 | 1.4 | 2.3 |
| *S. aureus* | PE+/FITC+ (%) | 22.7 | 25.8 | 34.1 | 25.4 | 30.3 |
| PE+/FITC- (%) | 3.9 | 4.6 | 2.3 | 3.3 | 2.9 |
| PE-/FITC+ (%) | 1.8 | 0.7 | 2.6 | 2.4 | 2.7 |