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AGGLUTINATION OF OYSTER (*CRASSOSTREA VIRGINICA*) HEMOCYTES FROM FIVE DIFFERENT REGIONS EXPOSED TO THE *LATHYRUS ODORATUS* LECTIN IN THE ABSENCE OF *HAPLOSPORIDIUM NELSONI* (PROTISTA)

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ABSTRACT: Hemocytes from oysters, *Crassostrea virginica*, not infected with *Haplosporidium nelsoni* collected from five locations along the Atlantic coast of USA were exposed to nine concentrations of the *Lathyrus odoratus* lectin. This resulted in the agglutination of varying percentages of hemocytes indicating the presence of lathyrose or a functionally very similar molecule on their surfaces. The agglutination was not inhibited by either D(+)- glucose or D(+)- mannose, which is characteristic of the *L. odoratus* lectin - lathyrose interaction. The relatively high percentages of clumped hemocytes in uninfected oysters further support the hypothesis that the presence of lathyrose is in some way associated with resistance of oysters to *H. nelsoni*.

KEY WORDS: Oyster, *Crassostrea virginica*, U.S.A., lathyrose, innate resistance marker, *Haplosporidium nelsoni*, protistan pathogenic parasite.

INTRODUCTION

As a result of an earlier study (CHENG, DOUGHERTY & BURRELL, 1994), it is known that there is a high degree of correlation between the presence of «lathyrose», an uncharacterized saccharide that binds to the *Lathyrus odoratus* lectin on the surface of hemocytes of the oyster *Crassostrea virginica* and the absence of the protistan parasite *Haplosporidium nelsoni*. The binding of lathyrose to the *L. odoratus* lectin is not inhibited by D(+)– glucose, D(+)- mannose, or a-methyl-D-mannoside, the known inhibitor saccharides for this lectin (TICHA, ZEINEDDINE & KOCOUREK, 1980).

During an extensive survey of oysters from several locations along the Atlantic coast of USA, although *H. nelsoni* was found in many of the oysters, uninfected ones also occurred. In order to provide further evidence for the correlation between the presence of lathyrose and the absence of *H. nelsoni*, the present study was carried out.

MATERIAL AND METHODS

Oysters: The uninfected oysters included in this study were collected from five sites: Gloucester, on the James River, which flows into the Chesapeake Bay, in Virginia; Solomons on the Chesapeake Bay in Maryland; May River (Beaufort County), and Folly Creek (Charleston County), both of which flow into the Atlantic Ocean, in South Carolina; and Port Norris, New Jersey, on Delaware Bay. The hemocytes from 144 uninfected oysters were subjected to the lectin tests reported herein: 7 from Solomons, 44 from Gloucester, 41 from Port Norris, 30 from May River, and 22 from Folly Creek. All of the oysters were ascertained to be uninfected by employing the panning technique of FORD *et al.* (1990) involving 1 ml of whole hemolymph.

Collection of hemocytes: Approximately 2.5 ml of whole hemolymph were collected from the adductor muscle sinus of each of the oysters by use of sterile 21 gauge hypodermic needles and 1-ml tuberculin syringes. One ml was used for «panning» and the remaining 1.5 ml samples were each washed 3 times with isotonic (540 mOsm) saline (IS) involving centrifugation at 300 *g* in a table top centrifuge. After the third wash, the cell pellets were gently resuspended in 2 ml of IS. The final cell counts averaged 1-1.5 × 10^4/ml.

Lectins: The most concentrated solution of the *L. odoratus* lectin employed was 0.1 mg/ml. The purified lectin, as well as D(+)– glucose and D(+)– mannose, the known inhibitor saccharides for this lectin (TICHA, ZEINEDDINE & KOCOUREK, 1980), were purchased from Sigma (St. Louis, Missouri, USA). The lectin solutions were prepared in phosphate-buffered saline and were serially diluted 2-fold with IS in microtiter plates to give final dilutions of 1:1 to 1:256. The agglutination tests were carried out in 96 well U-bottom plates (Cell Wells, Corning, New York, USA). To test for possible inhibition of hemocyte agglutination by D(+)- glucose or D(+)- mannose, the most concentrated lectin solution was serially diluted in 0.2 M solutions of the two saccharides.

Fifty µl of hemocyte suspension was added to each experimental and control well and the plates were incubated for 24 hr at room temperature (25° C). All of the hemocyte samples tested were from single oysters. In addition to the 144 oysters collected from the five areas mentioned, the cells from two additional oysters from each of the five regions were employed in negative control tests, i.e., IS, instead of lectin, was used. None of these resulted in agglutination of hemocytes.

In addition to the *L. odoratus* lectin, concanavalin A, type III (Con A) was included in every test as a positive control as it is known that it will agglutinate *C. virginica* hemocytes (YOSHINO, RENWRANTZ & CHENG, 1979; CHENG *et al.*, 1980; KANALEY & FORD, 1990; CHENG, DOUGHERTY & BURRELL, 1993). Con A was also purchased from Sigma as were D(+)- mannose and N-acetyl-D-glucosamine, the inhibiting saccharides employed. The most concentrated solution of Con A tested was 1.0 mg/ml, while to test the inhibitory effects of the two saccharides, the most concentrated Con A solution was serially diluted in 0.2 M solutions of the two sugars.

As CHENG *et al.* (1980) and CHENG, DOUGHERTY & BURRELL (1993) have demonstrated that not all of the hemocytes exposed to

selected lectins agglutinated, we determined the percentages of clumped and single cells at the highest concentration of *L. odoratus* lectin tested and at the 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256 concentrations. The counting of agglutinated and single cells was achieved with phase-contrast microscopy. When three or more cells were clumped, these were considered to be agglutinated. Pairs were seldom observed.

RESULTS

Solomons oysters: As indicated in Fig. 1, lathyrose is present on the surface of hemocytes of oysters from Solomons, Maryland. Specifically at the 1:1 concentration of the *L. odoratus* lectin, the mean number of agglutinated hemocytes was 60,25/100 cells. As the concentration of the *L. odoratus* lectin was reduced, so did the number of agglutinated cells (Fig. 1).

May River oysters: The pattern portrayed by hemocytes from May River oysters follows the same pattern as these of Solomons hemocytes when exposed to the *L. odoratus* lectin except that the percentages of agglutinated cells is less than in the case of Solomons oyster cells (Fig. 1).

Folly Creek oysters: The pattern exhibited by Folly Creek oyster hemocytes is essentially identical to that portrayed by May River oyster cells when exposed to the nine concentrations of the *L. odoratus* lectin (Fig. 1).

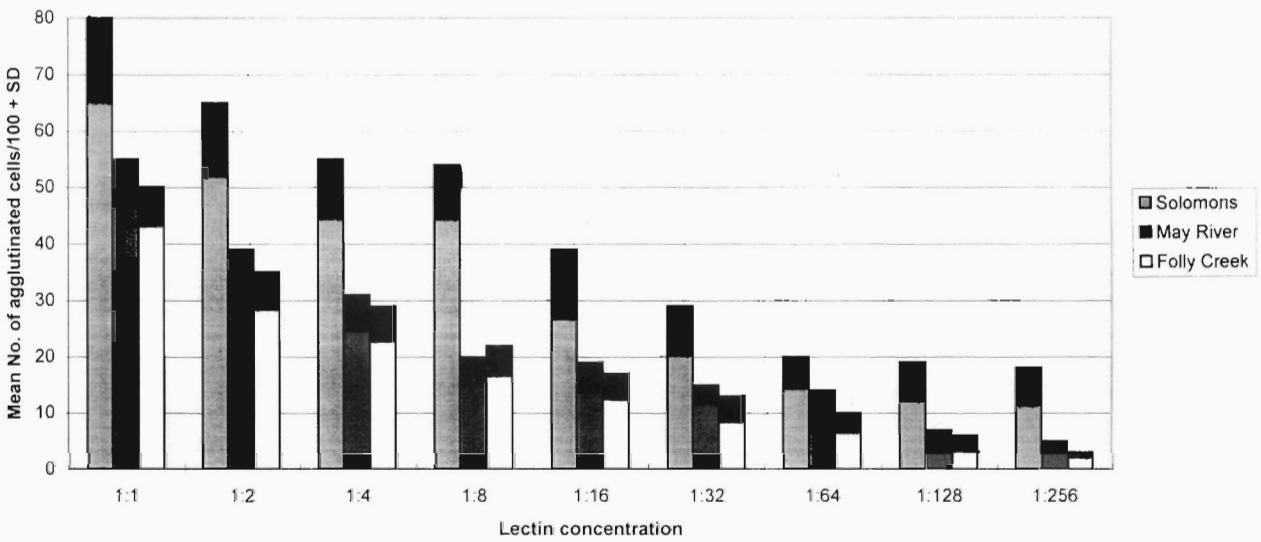

Fig. 1.– Solomons, Maryland vs. May River, South Carolina vs. Folly Creek, South Carolina oyster hemocytes exposed to *Lathyrus odoratus* lectin.

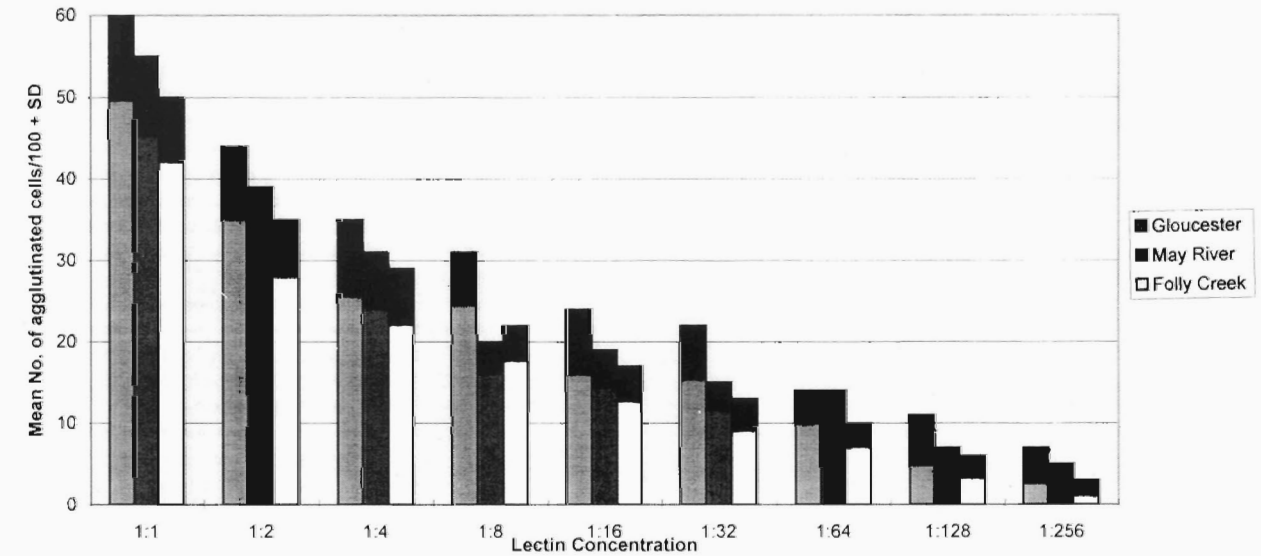

Fig. 2.– Gloucester, Virginia vs. May River, South Carolina vs. Folly Creek, South Carolina oyster hemocytes exposed to *Lathyrus odoratus* lectin.

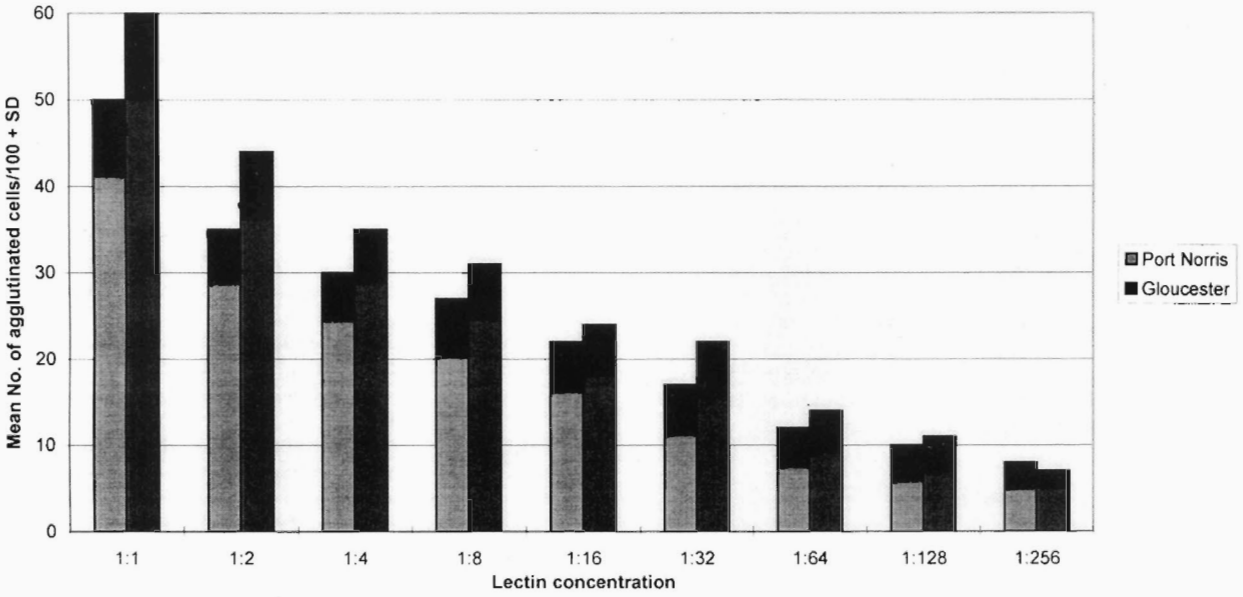

Fig. 3.– Port Norris, New Jersey *vs.* Gloucester, Virginia oyster hemocytes exposed to *Lathyrus odoratus* lectin.

That the saccharide on the surface of hemocytes that reacts with the *L. odoratus* lectin is lathyrose or a functionally very similar molecule is supported by the fact that neither D(+)– glucose nor D(+)– mannose exerted an inhibitory effect. This is characteristic of the *L. odoratus* lectin - lathyrose interaction (CHENG, DOUGHERTY & BURRELL, 1994).

Gloucester, May River, and Folly Creek oysters: When the percentages of agglutinated cells from Gloucester, Virginia, May River, South Carolina, and Folly Creek, South Carolina, oysters exposed to the nine concentrations of the *L. odoratus* lectin are compared, the results are comparable (Fig. 2).

Port Norris and Gloucester oysters: When the percentages of agglutinated hemocytes from uninfected Port Norris, New Jersey, oysters are compared to these from Gloucester, Virginia, oysters at the nine concentrations of the *L. odoratus* lectin (Fig. 3), the percentages of agglutinated hemocytes from both groups of oysters are comparable (Fig. 3).

DISCUSSION

It has been shown that approximately 23,1% of the oysters from Solomons, Maryland, were not infected with *H. nelsoni*, 50% of the oysters from Port Norris, New Jersey, were not infected, 27,2% of the oysters from May River, South Carolina, and 63% of the oysters from Folly Creek, South Carolina, were not infected with *H. nelsoni* (unpublished data). Based on a semi-quantitative scale (i.e., ++++ extremely heavy infection, +++ heavy infection, ++ medium infection, + light infection), both the May River and Folly Creek oysters infected with *H. nelsoni* harboured + to ++ infections. This correlates well with the results depicted in Fig. 1, i.e., there is a higher level of reactive lathyrose on the hemocytes of uninfected oysters from Solomons than on the cells of uninfected oysters from both May River and Folly Creek. This may reflect the fact that uninfected Solomons oysters are more resistant to *H. nelsoni*.

The earlier survey also revealed that 39,3% of the oysters from Gloucester, Virginia, were not infected with *H. nelsoni*. Furthermore, among the oysters harbouring *H. nelsoni*, the infection intensity at this site was + to ++ while, as stated, these in May River and Folly Creek oysters were also + to ++. This may be the reason why there are no significant differences in the percentages of agglutinated hemocytes from uninfected Gloucester, May River, and Folly Creek oysters when exposed to the nine concentrations of the *L. odoratus* lectin (Fig. 2).

As depicted in Fig. 3, there is no difference between the numbers of agglutinated hemocytes from uninfected Port Norris, New Jersey, and Gloucester, Virginia, oysters when exposed to the nine concentrations of the *L. odoratus* lectin. Gloucester oysters revealed 60,7% infection and the intensity was + to ++. Among Port Norris oysters, 50% were infected and the intensity of infection was also + to ++. That the infection intensities in these two groups of oysters is approximately the same may reflect the similarity between the percentages of agglutinated cells when exposed to the nine concentrations of the *L. odoratus* lectin (Fig. 3).

In view of the results presented herein, there is the suggestion that there are different strains of *C. virginica* relative to resistance to *H. nelsoni*. Specifically, it appe-

ars that the greater quantity of lathyrose, therefore, the higher percentage of agglutinated hemocytes when exposed to the *L. odoratus* lectin, is a characteristic of uninfected oysters from such heavily endemic areas as Solomons, Maryland. Perhaps these oysters are uninfected because of the higher amounts of lathyrose on their hemocyte surface; however, it remains undetermined how the presence of lathyrose is mechanistically associated with resistance of oysters to *H. nelsoni*.

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