Antibacterial and antifungal activities of polychaete Perinereis cultrifera

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Water, methanol and acetone extracts of polychaete Perinereis cultrifera were screened for their antibacterial and antifungal activities. Extracts were obtained from the whole body tissue of the animals and tested against 10 different pathogenic bacteria and 4 pathogenic fungi. Fractionated extracts of acetone (A), methanol (M) and acetone with methanol prepared in different concentrations such as 1, 5, 25, 50, 100, 200, mg/l were also tested against bacterial pathogens. Among the extracts, methanol showed maximum antibacterial activity against Staphylococcus aureus (8.0 mm) and minimum against Klebsiella oxytoca (1.0 mm) both in methanol and acetone extracts. As regards antifungal pathogens, methanol extract showed maximum activity against Rhizopus sp. (12.0 mm) and minimum against Aspergillus niger (2.0 mm) in the water extract. Trace activity was noticed against Mucor spp. and A. niger in water and acetone extracts respectively.

[Keywords: Antibacterial & antifungal activity, Perinereis cultrifera, Vellar estuary]

Introduction

Studies on antibacterial activity in marine organisms have been in vogue since many decades. Molluscs and echinoderms are probably the most extensively studied groups among the invertebrates. Added to these groups, annelids especially polychaete worms have gained prominence in this line of study in view of its bioactivity and structurally intriguing compounds. A review of literature on antibacterial activity of annelids particularly polychaetes revealed that only a sketchy information is available. On the contrary, a large number of works is available in other groups of invertebrates. Chain and Anderson studied on the antibacterial activity of the coelomic fluid from the polychaete Glycera dibranchiata and extracted the active molecules which showed the antibacterial activity against Gram-negative rod Serratia marcescens. Similarly, Lovell et al. described the antibacterial activity from volatile halogenated secondary metabolites produced by the polychaetes. Benkendorff et al. tested the antibacterial activity in 4 species of polychaetes against 4 marine bacterial pathogens namely Enterococcus sericolica, Vibrio anguillarum, V.alginolyticus and V. harveyi. Considering the paucity of information in this line, an attempt was made presently with a view to evaluate the potential antibacterial and antifungal activities of polychaete Perinereis cultrifera.

Materials and Methods

Perinereis cultrifera was collected in good numbers from the intertidal region of Vellar estuary, (Lat.11°49’ E; Long. 79° 46’ N) southeast coast of India. The potential antibacterial and antifungal activities were examined by standard methods. They were washed with distilled water. Extraction was done with three different solvents such as water, methanol and acetone. Ten species of pathogenic bacteria namely Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Lactobacillus vulgaris, Proteus mirabilis, Pseudomonas aeroginosa, Salmonella typhi, S. paratyphi, Staphylococcus aureus and Vibrio spp. were used to screen the antibacterial activity of the polychaete extracts. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Pathogens were swabbed on the surface of the Muller Hinton agar plates and discs (Whatman No. 1 filter paper with 9 mm diameter) impregnated with the 50 µl of polychaete extracts placed on the surface. All the three extracts were also fractionated and elutions were made with acetone (A), methanol (M) and acetone mixed with methanol in various proportions (E:M 18:2, 16:4, 12:8, 10:10, 8:12, 8:12, 6:14 and 2:18). Eluted fractions were assayed for antibacterial activity following the aforementioned disc diffusion method. To estimate the minimum inhibitory concentrations of extracts,
different concentrations such as 1, 5, 25, 50, 100, 200 mg/l were prepared and they were tested against the pathogens following the well diffusion agar method. After 24 h of incubation, the plates were removed and observations were made for inhibition zone against the pathogens.

The antifungal activity of the crude extracts was studied following the same method as that of antibacterial activity. The Discs (Whatman No. 1 filter paper with 9 mm diameter) impregnated with the 50 µl of polychaete extracts were placed in mycological agar plates seeded with four different strains (Aspergillus niger, A. flavus, Mucor spp. Rhizopus spp.) The cultures were incubated for 24-48 h at room temperature to obtain the maximum growth in the culture media. Control discs were also placed with water and solvents to assess the effect of water and solvents on pathogens.

Results and Discussion

The results of antimicrobial activity of different extracts of P. cultrifera are given in Table 1 and Plate 1. The results revealed that the water extract showed highest activity (4 mm) against E. coli and S. typhi and the lowest against L. vulgaris (2 mm), S. paratyphi and S. aureus (2 mm). In methanol extract, S. aureus exhibited the highest activity (8 mm) and P. mirabilis the lowest activity (2 mm). In acetone extract too, highest activity was noticed against E. coli (3 mm) and L. vulgaris (3 mm). Among the three extracts, methanol showed pronounced activity against S. aureus (8 mm) compared to other two extracts. Trace activity was observed against Vibrio spp. in water and methanol extracts, K. oxytoca in water and P. aeruginosa in all the three extracts while K. pneumoniae was found to be resistant to all the three extracts.

The chromatographic fractions of extracts showed interesting results. Of 11 fractions, almost all the fractions exhibited activity against bacterial pathogens. The fraction Acetone: Methanol 2:18 showed highest activity (9 mm) against E. coli, followed by, the fraction 10:10 showed next best activity (8 mm) against E. coli. The other fractions showed very weak activity against the pathogens (Table 2).

The results of Minimum Inhibitory Concentrations of various extracts revealed that, among the concentrations, the 25 mg concentration and above, in all the three extracts, showed activity against pathogens barring 5 mg concentration in acetone which showed activity against only 3 pathogens namely K. pneumoniae, P. aeruginosa and Vibrio spp. (Table 3).

In the present study, the extracts showed antibacterial activity against most of the pathogens barring a few. In a study conducted by Lovell and others on antibacterial activity of halogenated volatile secondary metabolites produced by polychaetes revealed that these metabolites are known to affect the rate of respiration. Similarly, Benkendorff et al. studied on the marine molluscs for their antibacterial activity against four marine
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pathogens, namely, *Enterococcus sericolicida*, *V. anguillarum*, *V. alginolyticus* and *V. harveyi*. Their results revealed that the egg masses of marine molluscs were found to inhibit the growth of two bacterial strains. Further they were also tested against three human pathogenic bacteria, such as *E. coli*, *S. aureus* and *P. aeruginosa* and found that the pronounced activity exhibited against *E. coli* is similar to the results of the present study. Chain and Anderson studied the antibacterial activity of coelomic fluid from the polychaete *Glycera dibranchiata* against few bacterial pathogens. Prem Anand et al. studied the antibacterial activity of common marine molluscs from Parangipettai coast and reported that the methanol extract of molluscs exhibited significant activity against *E. coli* (5.0). These findings corroborate the results of the present study since methanol extract of the present study also showed pronounced activity against *E. coli* (8 mm). Similarly, Emerson-Kagoo and Ayyakkannu studied the antibacterial activity in *Chicoreus ramosus* and got similar results.

Of the three extracts, which tested for antifungal activity, methanol extract showed the highest activity against *Rhizopus* spp. (12 mm) and the minimum was recorded against *Aspergillus niger* (2 mm) in water extract (Table 4 and Plate 2). Similar to this, Bhosal et al. reported that the methanol extract of polychaete *Sabellaria cementifera* showed maximum activity against *A. flavus* and minimum against *A. niger* in their study.

Thirumaran and Anantharaman studied the antifungal activity of seaweed *Hydroclathrus clathratus* and Padmakumar and Ayyakkannu screened microalgae for their antifungal activity. Abraham et al. studied on the antibacterial and antifungal activities of alcoholic extracts of holothurians species and found that among the
Plate 2—Antifungal activity in water, methanol and acetone extracts of *Perinereis cultrifera*

<table>
<thead>
<tr>
<th>Fungal Strains</th>
<th>Water (mm)</th>
<th>Methanol (mm)</th>
<th>Acetone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>2.0</td>
<td>3.0</td>
<td>Trace</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>6.0</td>
<td>11.0</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Mucor sp.</em></td>
<td>-</td>
<td>10.0</td>
<td>3.0</td>
</tr>
<tr>
<td><em>Rhizopus sp.</em></td>
<td>4.0</td>
<td>12.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

bacteria, as observed presently, *E. coli* and *S. typhi* showed highest activity and *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were found to be resistant in alcoholic extract. Barring these works, as there were not many works in this line, the results of the present study could not be discussed at length. Even though the present study revealed a wide spectral antibacterial and antifungal activity against few pathogens, extensive study is needed in this line in
order to develop potential compounds from this interesting group of organisms.

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References