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Polyunsaturated fatty acid-producing marine thraustochytrids: A potential source for antimicrobials

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ABSTRACT

Objective: To evaluate the antimicrobial activity of marine thraustochytrids extracted from different solvents.
Methods: Crude extracts were derived from marine thraustochytrids, isolated from decaying mangrove leaf litter. The extracts were tested for antibacterial activity by using agar disc diffusion method against 10 clinical bacterial strains. The extracts were also analysed for presence of functional chemical groups by using Fourier transform infrared spectroscopy.
Results: Thraustochytrid extracts exhibited potent antibacterial activity against both Gram-negative and -positive bacteria. The antibacterial activity was observed prominently in butanol extract, followed by petroleum ether, methanol and chloroform extracts. The antibacterial activity was maximum [(21.33 ± 1.52) mm] against *Staphylococcus aureus* and minimum [(7.00 ± 2.00)] mm against *Klebsiella pneumonia* and *Salmonella typhi*.
Conclusions: Thraustochytrids isolated from decaying mangrove leaf litter are potential sources of antibacterial compounds against clinical pathogens, which are called for further investigation of thraustochytrids as natural antibiotics.

1. Introduction

Today, infectious diseases are the major cause of death in developing countries and they hold the second position after heart diseases in the world. For instance, *Staphylococcus aureus* (*S. aureus*) can cause a range of illnesses from minor skin infections and sometimes it may cause life-threatening diseases such as pneumoniae and meningitis. Moreover, the pathogenic bacteria develops quick resistance to commercial antibiotics[1]. The advent of multiple resistant mechanism has limited the use of many major classes of antimicrobial compounds. The demand for efficient and non-toxic antibacterial therapeutics has been increasing with the increased incidence of bacterial infections. There is a growing interest in discovering new antimicrobial compounds with fewer environmental and toxicological risks and no resistance developed by the pathogens[2]. In this regard, fungi are targeted as one of the most suitable resources for reducing or eliminating pathogens. Among marine fungi, those living in association with mangrove sediment and detritus are a promising source of natural products and antimicrobial substances[3,4]. However, thraustochytrids are rarely attempted for their potential for antimicrobials[4-9].

Mangroves are one among the most productive detritus-based ecosystem[10,11]. Fungi are extremely important in litter decomposition of mangrove habitats[12-14]. Thraustochytrids species are found abundantly in decomposing mangrove leaf litter[15,16]. The fungi, including thraustochytrids produce many hydrolytic enzymes and a variety of bioactive compounds to supply nutrients associated with organisms during leaf litter decomposition[8,16-21]. In order to compete with other microbes for survival and multiplication, thraustochytrids are required to produce antimicrobial substances. However, studies on antimicrobial activity of thraustochytrids are scanty and hence, the present study was undertaken to evaluate the antibacterial potential of thraustochytrids against clinical human pathogens.

2. Materials and methods

2.1. Microorganisms and culture conditions

A pure strain of thraustochytrids isolated from decaying mangrove

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leaf litter in our laboratory was used for the present study[22]. This strain was inoculated in a production medium containing 3 g glucose, 1.25 g yeasts and 1.25 g peptone in one liter of 50% seawater at pH 7.2 and incubated at 28 ˚C for 7–9 days. Then the biomass was harvested for extraction of intracellular metabolites and lyophilized.

2.2. Preparation of solvent extraction

The lyophilized thraustochytrid strain was extracted in chloroform, methanol, *n*-butanol and petroleum ether, separately in the ratio of 2:1[23,24]. The extracts were obtained by grinding the sample in a pestle and mortar and filtering through the Whatman No. 1 filter paper. The filtrate was centrifuged at 3000 r/min for 10 min. The supernatant was collected and stored (-4 ˚C) for further studies.

2.3. In vitro antibacterial activity of metabolites

Crude extracts were tested for antibacterial activities by agar disc diffusion method[24,25]. The activity was tested against a total of 10 clinical bacterial strains: *Escherichia coli* (*E. coli*), *Klebsiella oxytoca* (*K. oxytoca*), *Klebsiella pneumonia* (*K. pneumonia*), *Proteus mirabilis* (*P. mirabilis*), *Salmonella paratyphi* (*S. paratyphi*), *Salmonella typhi* (*S. typhi*), *S. aureus, Streptococcus pyogens* (*S. pyogens*), *Vibrio cholerae* (*V. cholerae*) and *Vibrio parahaemolyticus* (*V. parahaemolyticus*), obtained from Raja Muthiah Medical College, Annamalai University. All the experiments were performed in triplicate.

2.4. Fourier transfer infrared spectroscopy (FTIR) analysis

FTIR spectral analysis of crude butanol extract of thraustochytrid was made, by mixing 10 mg of samples with 100 mg of dried potassium bromide and compressed further to prepare a salt disc (10 mm) for the spectrum reading at a range between 500 cm^{-1} and 4000 cm^{-1}.

2.5. Statistical analysis

The results obtained from the experiment were expressed as mean ± SD. One-way ANOVA was made using SPSS-16 version software followed by Duncan's multiple range test. $P < 0.05$ was considered for describing the significant levels.

3. Results

3.1. Antibacterial activity

The strain of thaustochytrid (Figure 1) was extracted in four organic solvents (chloroform, methanol, *n*-butanol, petroleum ether). The crude extracts were tested against 10 clinical bacterial pathogens. The results were summarized in Table 1. The maximum activity (21.33 ± 1.52) mm was recorded against *S. aureus* and the minimum activity (7.00 ± 2.00) mm was against *K. pneumonia* and *S. typhi*. Most prominent antibacterial activity was recorded with *n*-butanol extract, followed by petroleum ether, methanol, chloroform and distilled water extracts (Table 1).

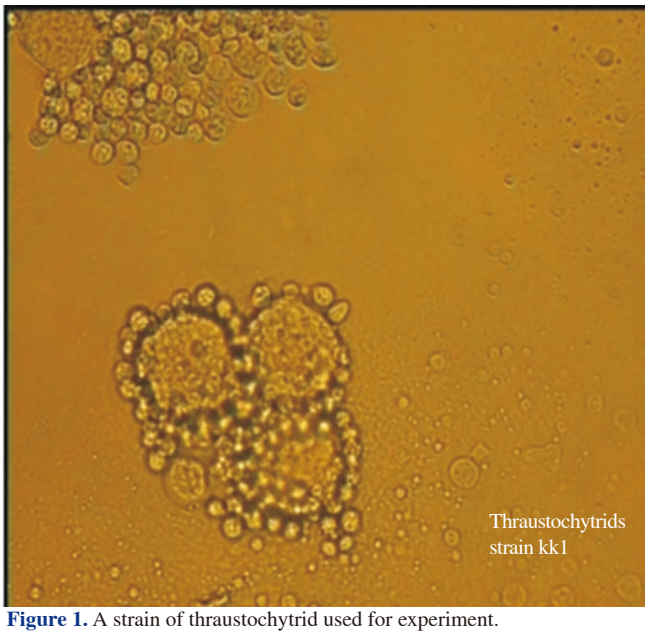

Figure 1. A strain of thraustochytrid used for experiment.

Table 1
Antibacterial activity of (indicated by inhibition zones) extracts of a thraustochytrid strain against clinical pathogens. mm.

Clinical pathogens	Zone of inhibition (mean ± SD)				Average
	Chloroform	Methanol	*n*-Butanol	Petroleum ether	
E. coli	9.33 ± 2.51	13.66 ± 2.51	9.00 ± 2.00	14.00 ± 1.73	11.49
K. oxytoca	9.33 ± 2.08	9.33 ± 2.51	7.33 ± 1.52	12.00 ± 2.00	9.49
K. pneumonia	7.33 ± 1.52	7.00 ± 2.00	8.66 ± 2.08	11.66 ± 0.57	8.66
P. mirabilis	8.66 ± 2.08	9.00 ± 2.00	9.66 ± 1.52	13.66 ± 1.52	10.24
S. paratyphi	10.00 ± 1.00	8.00 ± 1.73	9.33 ± 2.51	12.66 ± 1.52	9.99
S. typhi	8.00 ± 1.73	7.00 ± 2.00	13.33 ± 1.52	15.66 ± 1.52	10.99
S. aureus	10.66 ± 2.08	11.66 ± 0.57	21.33 ± 1.52	12.33 ± 1.15	13.99
S. pyogens	10.00 ± 2.00	8.33 ± 1.52	11.00 ± 2.00	8.33 ± 1.52	9.41
V. cholerae	11.33 ± 1.52	9.33 ± 2.30	10.33 ± 2.08	11.00 ± 2.00	10.49
V. parahaemolyticus	7.66 ± 1.15	8.00 ± 1.73	10.66 ± 0.57	10.66 ± 1.52	9.24

3.2. Spectroscopic (FTIR) characteristics of the antimicrobial agent

The results of FTIR spectroscopic analysis were shown in Figure 2 and Table 2. The peaks between 1650 and 1660 cm^{-1} were generally assigned to α-helical absorption[26,27]. One peak at 3419.79 cm^{-1} and another one peak at 1649.14 cm^{-1} indicated two types of α-helices present in the sample. The relative intensities of the amide I peaks revealed the secondary structure of the protein. One peak at 1404.18 cm^{-1} was associated with $\delta_s(CH_3)$ of proteins. The peaks between 2700 and 3300 cm^{-1} were generally assigned to lipids. The peak at 2931.80 cm^{-1} indicated C-H stretch present in the sample. The peaks between 1020 and 1250 cm^{-1} were generally assigned to amide III: β-sheet/aliphatic amines. The peak at 1242.16 cm^{-1} indicated C-N stretch present in the sample. The peak at 1058.92 cm^{-1} was generally assigned to carbohydrate for the presence of C-OH groups in the sample. The peak at 1105.21 cm^{-1} indicated OH group of ribose rings of RNA present in the sample. The peaks between 500 and 670 cm^{-1} were generally assigned to amide VII. The peak at 561.29 cm^{-1} indicated the presence of C-Br in the sample[28,29].

Table 2
FTIR band assignments for functional groups found in the spectra of thraustochytrid extract.

Functional groups	Wave number value (cm^{-1})
Amide I: C=O stretch	3419.79
(C-H) from methylene (-CH_2) groups of lipids	2931.80
(C-H) from methyl (-CH_3) groups of lipids	
Amide I: α-helix	1649.14
Amide II: perpendicular modes of the α-helix and antiparallel β-sheet	1554.63
$\upsilon_s(COO^-)$ associated with $\delta_s(CH_3)$ of proteins	1404.18
Amide III: β-sheet	1242.16
υ_s(C-O) at the 20-OH group of ribose rings in RNA	1105.21
$\upsilon_s(PO_2^-)$ of the phosphodiester backbone of nucleic acids (DNA and RNA) and phospholipids	
υ_s(R-O-P-O-R0) from ring vibrations of carbohydrates	1058.92
υ_s(C-O) coupled with δ(C-O) of C-OH groups of carbohydrate	
Amide VII (C-Br)	561.29

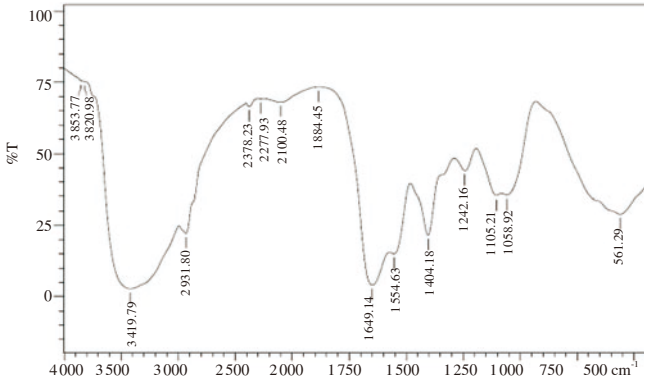

Figure 2. FTIR spectrum of thraustochytrid extract.

4. Discussion

In the present study, the crude extracts of a thraustochytrid strain showed antibacterial activity against the two Gram-positive bacteria (*S. aureus* and *S. pyogens*) and the eight Gram-negative bacteria (*E. coli*, *K. oxytoca*, *K. pneumonia*, *P. mirabilis*, *S. paratyphi*, *S. typhi*, *V. cholerae* and *V. parahaemolyticus*). The thraustochytrid extract exhibited the highest antibacterial activity in terms of inhibition zone (21.33 ± 1.52) mm against *S. aureus*. This inhibitory activity might be due to the destruction of bacterial cell wall and hydrolytic enzymes, produced by thraustochytrids[16]. The antibacterial activity can also be attributed to fatty acids such as linoleic acid, which are proved to have antibacterial, antiviral, antitumor, antiinflammatory, antiprolierative and antioxidant activities[30-33].

Marine filamentous fungi are rich in biologically active secondary metabolites with tremendous potential as a source of new medicines even at low concentrations of their secondary metabolites[34]. According to Cuomo *et al*., marine fungi have good activity profiles when compared to terrestrial fungi, making them a very promising source for the isolation of biologically active secondary metabolites[35]. Jayanthi *et al*. reported the antimicrobial activities of fungi with maximum inhibition to *K. oxytoca* and *V. cholerae*, and with minimum inhibition to *S. paratyphi* and *S. aureus*[36]. Joel and Bhimba have reported bioactive metabolites from a fungus, *Pestalotiopsis microspora* VB5 isolated from the leaves of *Rhizophora mucronata* and *Avicennia officinalis* from Pichavaram mangrove forest in southeast coast of India. This fungus shows antimicrobial activity against bacteria *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 by agar well diffusion method[37]. The antimicrobial activity of mangrove endophytic fungi is well-documented[7,9,38-40]. The antimicrobial activity of *Bacillus* species was found maximally against *K. oxytoca* and *V. cholerae*, and minimally against *S. paratyphi* and *S. aureus*. Bhimba *et al*. reported the antibacterial activity of marine-derived fungus *Phoma herbarum*, isolated from mangrove leaves[41]. The isolated fungi exhibited maximum activity in 100 μL concentration against *Micrococcus luteus* and *V. cholerae*. Phalate derivates from this fungus showed promising potential of antibacterial activity[41]. In the present study, the crude extract was analysed for functional groups of chemicals present, by using FTIR spectra. This revealed the presence of a typical amine group, methyl groups of protein, C-H stretching vibrations of lipids, C-N group, OH group of ribose rings and C-OH groups of carbohydrate. Similar results have been reported by earlier workers[28,40,42].

In the present study, a wide spectral antibacterial activity was recorded against the clinical pathogens. The maximum zone of inhibition was conferred by the *n*-butanol followed by petroleum ether, methanol and chloroform from the thraustochytrids extract. However, distilled water used as a negative control had no bacterial inhibition (data not shown). Further concentration of the active principle in the extract showed either bacteriocidal or bacteriostatic action against the bacteria, *i.e.* a low dose of a bacteriostatic antibacterial agent may only inhibit bacterial growth, while a high dose of a bactericidal antibacterial agent will destroy the microbial cells[43]. Our results suggest that the crude extracts of thraustochytrids can be considered as promising antimicrobials in controlling clinical pathogens. In order to produce the thraustochytrids extracts in large quantity and to render the bioprocess feasibility and practically, we are now attempting to isolate the active novel compounds from the crude extracts and determine their chemical structures. Further studies will fulfill for purification, structural elucidation and further evaluation in this field of study. Finally, we conclude that thraustochytrids from the mangrove biotope are potential sources of bioactive compounds and should be investigated for natural antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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