ORIGINAL ARTICLE

Screening and isolation of antibiotic-producing microbes from sea cucumber and testing their broad-spectrum activity

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Abstract

Background: Multidrug resistance of microbes forced the scientific community to search for newer antibiotics to treat infectious diseases. The literature review revealed that fishermen used the sea cucumber species for healing the wounds. **Aim:** As sea cucumber is rich in microbial flora, an attempt has been made to screen the antibiotic-producing microbes from them. **Materials and Methods:** Five sea cucumbers were collected from Kanyakumari district, Tamil Nadu, and dissected. The intestinal fluids and coelomic fluids were collected and named as IF and cystic fibrosis CF, respectively. The crowded plate method was followed to screen the antibiotic-producing microbes. As part of the primary screening, the perpendicular streak method was carried out to reveal the broad-spectrum potential of isolates. The selected isolates which showed the broad spectrum of activity were grown in soyabean casein broth media to produce the antibiotic principles. After the separation of cells from the fermented media, the agar well method was carried out against test organisms to reveal the broad spectrum of activity of the fermented broths. **Results:** The present investigation resulted in the isolation of 35 isolates with antibiotic-producing ability in crowded plate method. Out of 35 isolates, only 9 of them passed the primary screening. The secondary screening revealed that the fermented broths of three isolates (named IF_{32}, IF_{52}, and CF_{42}) were found to have a better broad spectrum of activity.

Key words: Antibiotic-producing, primary screening, screening and isolation, sea cucumber, secondary screening

INTRODUCTION

An increase in resistant infectious microbes forced the scientific community to search for newer antibiotics for the treatment of infectious diseases. Moreover, it becomes an ever-ending task for the researchers to screen the newer antibiotics for broad spectrum of activity. Synthetic molecules and natural sources are equally balanced options for newer antibiotic search.[1]

Isolation of antibiotic-producing microbes from soil has reached to an extent that scientist had ended up with repetitive isolation of the same molecules in the past decades.[2] Hence, search for other natural sources for antibiotic producers is going on.[3] Reports about sea cucumber being used by fishermen to treat the wound and also about the microbial flora of sea cucumber made us screen the same for antibiotic-producing microbes.[4-8]

This article mainly focuses on the work carried out on the microbial flora of sea cucumber species of the Indian coastal region to explore the antibiotic-producing capabilities. As part of the primary screening, crowded plate method and perpendicular streak method were carried out to screen the

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antibiotic potential of the microbial flora. Selected isolates were taken for secondary screening to access the broad-spectrum potentiality of the crude extracts.

METHODOLOGY

Collection of Microbial Flora of Sea Cucumber

Five sea cucumbers were collected from Kanyakumari district, Tamil Nadu, and transported live to the laboratory through plastic bags filled with water. The species were dissected in the laboratory to collect the intestinal fluids and coelomic fluids. The process was carried out under aseptic conditions to prevent contamination.[9-13] The collected intestinal fluids and coelomic fluids were named with IF and cystic fibrosis (CF), respectively, along with numbers in subscript to differentiate the collected fluids of each sea cucumber. (IF_1, IF_2, IF_3, IF_4, IF_5, CF_1, CF_2, CF_3, CF_4, and CF_5)

Primary Screening

Crowded plate method

Soyabean casein agar media were sterilized and transfer aseptically into the required number of sterile Petri plates. A hundred microliters of each collected fluid were aseptically transferred over the solidified soyabean casein agar media in the Petri plates separately. The spread plate method of inoculation was done using sterile "L" bend rod. Inoculated Petri plates were incubated at 28°C for 24 h. The colonies which produced a zone of inhibition (ZOI) were isolated and used for further studies.[2]

Perpendicular streak method

The isolates from the crowded plate method were separately inoculated in Mueller-Hinton agar media containing Petri plates by a single streak at the center of the media and incubated at 28℃ for 4 days. After 4 days of incubation, 24 h old cultures of *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 1610), and *Candida albicans* (MTCC 1637), having optical density (OD) of 1 at 540 nm, were seeded by streaking a single line of 5 cm length perpendicular to the streaked line of isolates. All the Petri plates were incubated for 2 days at 28℃.[2,13]

Secondary screening

The isolates, which showed moderate to good activity in the primary screening technique, were selected for Secondary screening. Soyabean casein broth media were used as a medium for fermentation of selected isolates. After fermentation, the cells were removed by filtration and the fermented broths were used for secondary screening. The secondary screening was performed against two Gram-positive, two Gram-negative, and two fungal species. Required number of Mueller-Hinton agar media was seeded with 24 h old cultures of test organisms (*Staphylococcus aureus* MTCC 1430, *B. subtilis* MTCC 121, *E. coli* MTCC 1610, *Pseudomonas aeruginosa* MTCC 2453, *C. albicans* MTCC 1637, *and Aspergillus niger* MTCC 8652) having OD of 1 at 540 nm by spread plate method of inoculation. Six-millimeter diameter containing wells were made in seeded Mueller-Hinton agar media aseptically using sterile borer. A hundred microliters of fermented broths of selected isolates were loaded in appropriately marked wells. All tests were performed in triplicate and average value has been taken for comparison and reporting.[2,13]

RESULTS

Primary Screening

Crowded plate method

The colonies which showed ZOI in crowded plate techniques are marked in Figure 1. Thirty-five colonies were found to produce ZOI and were isolated and stored in soyabean casein agar media and used for further studies.

Perpendicular streak method

Among the 35 isolates, only the isolates named CF_{11}, IF_{21}, IF_{22}, CF_{21}, CF_{24}, IF_{31}, IF_{32}, CF_{42}, and IF_{52} were found to inhibit the growth of perpendicularly streaked test organisms up to the expected level. These nine organisms were found to produce "moderate" to "good" activity against the test organisms. Obtained inhibition area on all three streaked test organisms of ≥1 cm but <2 cm was considered as "moderate" activity. The inhibition area of ≥2 cm was considered as "good." If the area of inhibition is <1 cm or the isolate is not showing activity against any of the test organism though shows higher activity on the remaining, then it was considered as "poor activity." As the remaining 26 isolates showed poor activity, they were discarded. Among the selected nine isolates, the IF_{32} and CF_{42} were found to produce almost complete inhibition (5 cm length) of streaked lines of test organisms.

Secondary screening

The ZOIs produced by fermented broths of selected isolates from primary screening against test organisms are given in Table 1. The IF_{32}, IF_{52}, and CF_{42} were found to produce ZOIs (values mentioned in Table 1) against two Gram-positive, two Gram-negative, and two fungal species. IF_{21} and IF_{22} were found to produce ZOI only against Gram-positive organisms. CF_{11} was found to be active against fungal species only. CF_{21} and CF_{24} were found to be ineffective against fungal species as they failed to produce ZOI against tested fungal species. Likewise, IF_{31} was failed to inhibit the growth of Gram-positive species.

DISCUSSION

The need of the hour for the treatment of multidrug resistance microbes is newer antibiotics. The search from the natural

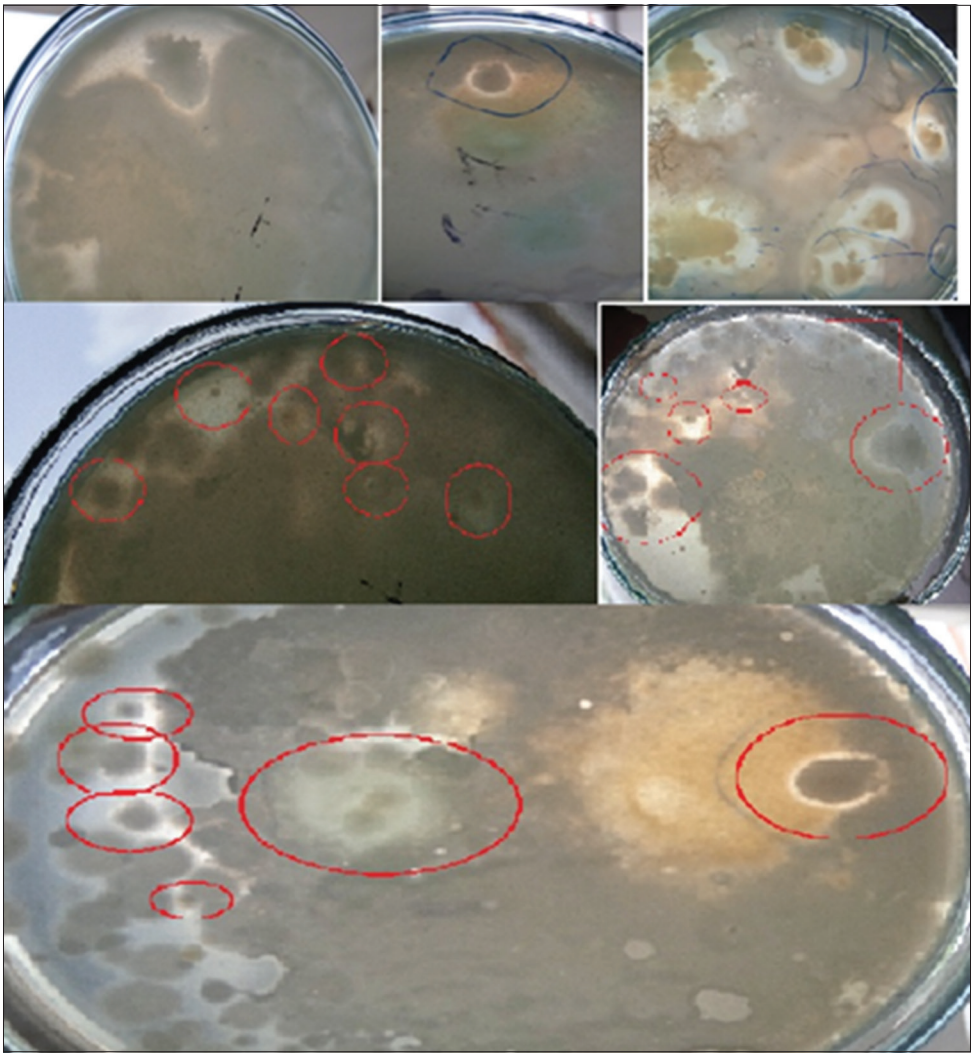

Figure 1: Colonies showing zone of inhibition in the crowded plate method

Table 1: Zone of inhibition produced by selected isolates in secondary screening

Name of the isolate	Zone of inhibition (mm)					
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***	***Aspergillus niger***
IF_{21}	12	9	-	-	-	-
IF_{22}	15	12	-	-	-	-
IF_{31}	-	-	18	16	18	17
IF_{32}	26	22	21	23	19	16
IF_{52}	28	27	26	31	21	19
CF_{11}	-	-	-	-	10	13
CF_{21}	13	15	14	18	-	-
CF_{24}	9	10	12	11	-	-
CF_{42}	25	22	19	18	19	17

mm: millimeter

source already resulted in Nobel Prize-winning molecules (penicillin and streptomycin). Hence, the thirst for newer antibiotics from the natural source is ever ending among the scientists.

The investigation started with the clue of wound healing property of sea cucumber species, resulted in the identification of 35 colonies producing ZOI in the crowded plate method. Among the nine of the isolates, IF_{21}, IF_{22}, CF_{11}, CF_{21}, CF_{24},

IF_{31}, IF_{32}, CF_{42}, and IF_{52}, were found to pass the primary screening method as they showed moderate to good activity against test organisms. Secondary screening revealed that the organism named IF_{32}, $IF_{52,}$ and CF_{42} was only found to produce antibiotic principles with broad spectrum of activity as the fermented broths produced ZOI against all the test organisms. Remaining isolates were found to produce either of the narrow spectrum or of antifungal molecules only.

CONCLUSION

The isolated microbes are found to have the potential to produce broad-spectrum antibiotics in our screening studies. Further, work will be carried out to isolate the antibiotic principle in pure form and to characterize the isolated microbes for the possible industrial applicability.

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