

Optimization of Temperature, pH and Duration for Enhanced Antibiotic Productivity of Microbes Isolated from Sea Cucumber

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Abstract

Introduction: Five sea cucumber species were collected from fishermen at coastal region of Kanyakumari district, Tamil Nadu, and dissected aseptically. Intestinal fluids and coelomic fluids were collected. After serial dilution of the collected fluids, primary screening was carried out by crowded plate method using modified Soybean Casein Agar media (Kester *et al.*). Selected antibiotic-producing organisms producing zone of inhibition (ZOI) higher than 6 mm (ZOI >6 mm) were evaluated for broad spectrum of activity using two Gram positive, two Gram negative, and two fungal species by perpendicular streak method. Biochemical characterization and nutrient optimization were carried out for selected three isolates (named IF₃₂, IF₅₂, and CF₄₂) which were found to have broad spectrum of activity as well as passed Kirby–Bauer antimicrobial susceptibility test. This article focuses on optimization of temperature, pH, and duration for maximum antibiotic productivity of the selected isolates. **Methodology:** Optimum temperature, pH, and duration of maximum antibiotic production were evaluated for the selected antibiotic producers. Crude antibiotic was collected after 24 h of incubation under different temperature and pH conditions and tested against *Staphylococcus aureus* (MTCC 1430). The specific temperature and pH at which maximum ZOI was produced by the collected crude extracts against test organisms were considered. The duration of maximum antibiotic production was evaluated by incubating the isolated organisms under the optimized temperature and pH. **Results:** The optimum temperature for the production of crude antibiotic from IF₃₂, IF₅₂, and CF₄₂ was found to be 32°C, 35°C, and 31°C, respectively. The pH at which maximum antibiotic production was observed is found to be 8, 7, and 9, respectively, for IF₃₂, IF₅₂, and CF₄₂. The duration of maximum antibiotic productivity at the optimized conditions was found to be 4th, 5th, and 3rd days, respectively, for IF₃₂, IF₅₂, and CF₄₂.

Key words: Antibiotic production, microbial flora of sea cucumber, optimization of pH, optimization of temperature

INTRODUCTION

Antibiotic search is an ever-ending process due to the increasing number of resistance species. Many novel molecules such as penicillin, streptomycin, tetracycline, and erythromycin were isolated from soil microbes. Nowadays, soil screening process largely results in repetition of existing antibiotics.

Sea cucumber, is rich in microbes in its coelomic region and intestinal region, was reportedly used to treat wounds by fishermen. This clues

the possibility of antibiotic-producing organisms present in the sea cucumber, instead of sea cucumber itself, having the antibiotic principles.^[1-5]

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Received: 16-09-2019

Revised: 14-11-2019

Accepted: 26-11-2019

Out of primary and secondary screening of intestinal fluids and coelomic fluids of sea cucumbers, three microbial isolates (named IF₃₂, IF₅₂, and CF₄₂) were found to be mesophilic in temperature requirement, have a broad spectrum of activity against test organisms and also passed Kirby–Bauer antimicrobial susceptibility test. The isolated microorganisms are to be used for the production of antibiotics. As part of this process, the optimization of various parameters for the maximum production of antibiotic principle is an essential part of study. This article aims to evaluate the optimum temperature, pH, and the duration at which the isolates produce the maximum crude antibiotics.

METHODOLOGY

Media

The selected isolates were inoculated in 400 ml of optimized Soybean Casein Digest Media separately in Erlenmeyer flasks with a capacity of 500 ml. About 1% of 24 h old inoculums having optical density (OD) of 1 at 600 nm were used for inoculation. Three parameters, temperature, pH, and duration of maximum antibiotic production, were evaluated in this study.^[6]

Evaluation of optimum temperature

Three isolates were separately inoculated in sufficient number of Erlenmeyer flasks to incubate at various temperatures. The inoculated flasks were incubated at 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C, 37°C, 38°C, 39°C, and 40°C. The antibiotic productivity of the isolates was evaluated after 24 h against *Staphylococcus aureus* (MTCC 1430).^[7-9]

Evaluation of optimum pH

By keeping the optimum temperature for the maximum antibiotic productivity constant, the pH of the media was varied. Antibiotic productivity was tested at various pH of 4, 5, 6, 7, 8, 9, 10, 11, and 12 for selected isolates. The crude antibiotics were isolated after 24 h and tested against *S. aureus* (MTCC 1430).^[10-12]

Estimation of duration for enhanced production

The duration of enhanced antibiotic productivity was estimated by incubating the isolates at evaluated optimum temperature and pH conditions. The samples were collected at various time intervals up to 10 days and tested against *S. aureus* (MTCC 1430).^[6,9,13,14]

Evaluation of antibiotic productivity

The antibiotic productivity during optimization of temperature, pH, and duration was evaluated using test organism *S. aureus* (MTCC 1430). Four milliliters of sample were collected at specified time intervals. Collected samples were centrifuged at 12,000 rpm for 20 min. The filtrate was collected and filtered through 0.22 mm Millipore membrane filter. Twenty-four hours old cultures of test organism, having OD of 1 at 600 nm, were inoculated on Mueller-Hinton Agar media by pour plate method and required numbers of wells were made on the plate with the help of 6 mm sterile borer. Sixty microliters of the obtained filtrates were loaded into the well and the plates were incubated in the upright position for 24 h at 37°C. Zone of inhibition (ZOI) surrounding the wells was noted in millimeters and compared.^[9,10,12]

RESULTS

Effect of temperature

The antibiotic production observed from 28 to 36°C for IF₃₂. The maximum ZOI (16.4 mm) against the test organism was observed at 32°C. The optimum temperature for IF₅₂ and CF₄₂ was observed at 35°C and 31°C, respectively, and the ZOI observed is 19.5 mm and 13.4 mm. The antibiotic production for IF₅₂ starts from 29°C and continues till the higher range of temperature tested under study. The CF₄₂ is found to have the antibiotic production range from 27°C to 34°C. Table 1 shows the results of ZOI against the test organism produced

Table 1: Effect of temperature

Temperature in celsius	ZOI in mm (IF ₃₂)	ZOI in mm (IF ₅₂)	ZOI in mm (CF ₄₂)
26	0	0	0
27	0	0	8
28	8	0	8.9
29	9.2	7.9	10
30	12.6	8.5	11.2
31	13.8	10.1	13.4
32	16.4	11.5	10
33	15	13	9.3
34	14.6	16.4	7.5
35	10	19.5	0
36	8.2	18	0
37	0	14.5	0
38	0	12	0
39	0	11.1	0
40	0	9.5	0

ZOI: Zone of inhibition, mm: Millimeters

by the filtrates collected from selected isolates kept under various temperatures.

Effect of pH

The following Table 2 shows the ZOI produced by the filtrates obtained at tested pH range. IF₃₂ was found to show the maximum antibiotic productivity at pH 8 and ZOI produced against *S. aureus* was 17.6 mm. Maximum antibiotic production (ZOI – 18.6 mm) was observed at pH 7 by IF₅₂. The ZOI (24.3 mm) produced by the filtrate collected from CF₄₂ kept at pH 9 was found maximum.

Effect of incubation period on antibiotic production

IF₃₂ showed the highest antibiotic production on the 4th day though the production started after 24 h. Isolates IF₅₂ and CF₃₂ also started producing antibiotic principles from 24 h of incubation. However, they showed maximum antibiotic production on the 5th and 3rd days, respectively. The following Table 3 shows the ZOI produced by the filtrate collected at

various intervals during the 10 days of incubation of the selected isolates.

DISCUSSION

The results of this study reveal the optimum conditions required for the selected microbial isolates to produce maximum antibiotic principle. The isolate IF₃₂ is found to show maximum productivity of antibiotic principle at pH 8 and temperature 32°C in 4 days. Upon 5 days of incubation, at pH 7 and temperature 35°C, IF₅₂ produced the maximum antibiotic principle. The optimum conditions for CF₄₂ are found to be 31°C, pH of 9, and incubation period of 3 days for maximum antibiotic productivity.

CONCLUSION

The present work will be helpful to produce the antibiotic principles from the isolated microorganism in a maximum quantity. Further work will be carried out to purify and identify the nature of the antibiotic molecules.

ACKNOWLEDGMENT

The authors are thankful to Creative Educational Society's College of Pharmacy, Chinnatekur, Kurnool-518218, Andhra Pradesh, India, for providing facilities and support to complete this work.

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Table 2: Effect of pH

pH	ZOI in mm (IF ₃₂)	ZOI in mm (IF ₅₂)	ZOI in mm (CF ₄₂)
4	0	0	0
5	0	8.1	0
6	9.5	11.3	12
7	13.2	18.6	18.6
8	17.6	16.2	21
9	12	11	24.3
10	11.6	7.4	20.2
11	9	0	17.3
12	8.3	0	11.1

ZOI: Zone of inhibition, mm: Millimeters

Table 3: Effect of incubation period

Effect of incubation period (days)	ZOI in mm (IF ₃₂)	ZOI in mm (IF ₅₂)	ZOI in mm (CF ₄₂)
1	17.3	18.9	24
2	21	22.3	26.8
3	23.6	27.2	28.3
4	27.3	31.1	28
5	26.5	33.1	27.8
6	26	29.8	27.4
7	25.6	26.7	27.6
8	25.2	26.2	27.3
9	24	26	27
10	22.2	25.1	27

ZOI: Zone of inhibition, mm: Millimeters

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Source of Support: Nil. **Conflict of Interest:** None declared.