

PRODUCTION OF NOVEL AMYLASE ENZYME FROM HALICONA FIBULATA ASSOCIATED MARINE BACTERIA AGAINST UTI PATHOGEN

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ABSTRACT

Today, urinary tract infection is a major health problem affecting pregnancy women and its manifestation persists throughout the life despite treatment, it is a multifactorial disease which is caused by host agents and environmental factors. The UTI infection caused by *Escherichia coli* also most common pathogen in human life, is also serious problem among UTI patients. So midstream urine samples were collected from different Govt. Hospital in and around Tirupur District. A total of 20 multidrug resistant strains were isolated from 50 urine samples and were analyzed and characterized. Further, the antimicrobial susceptibilities of this isolates was assessed against 8 commonly used antibiotics, Nalidixic acid (5mcg), Cefotaxime (5mcg),

Clindamycin (10mcg), Vancomycin (30mcg), Ampicillin (10mcg), Chloramphenicol (10mcg), Tetracycline (30mcg) and Ceftriaxone (30mcg). Among these strains 70%, 80%, 50%, 75%, 70%, 10%, 40% and 85% were found to be exhibit a significance degree of resistance to four groups of antibiotics. Currently, the development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. During the last two decade, several studies have been performed to establish biologically active products from marine sources, which mostly deals with marine bacteria and their potential role in the production of metabolites is becoming thrust area for research, sponges have been recognized as a rich source of novel compounds that are of potential interest to mankind for diagnosis urinary tract infection. In this study marine sponge *Halicona fibulata* were isolated and identified using biochemical characterization. Further, the associated microbe recovered

from the sample and it was checked for production of amylase enzyme. The selected colony ability to produce the amylase enzyme and it was purified by ammonium sulphate precipitation, dialysis and gel filtration chromatography. Finally the different concentration of purified amylase enzyme 50µg, 100µg, and 150µg tested against the *E. coli*. The maximum zone of inhibition (22 mm) was observed against the isolate MT07 at 150µl. The minimum zone (16 mm) was recorded in the strain MT08. In this study sponge derived amylase enzyme was used and it shows prominent antimicrobial activity against UTI pathogen. Thus an alternative strategy targeting the endosymbiosis bacteria of sponges for the screening of bioactive natural products may prove to be an effective approach to come to blows emerging multi resistant clinical pathogens.

KEYWORDS: UTI pathogen, Antibiotic susceptibility, Marine Sponge, Amylase enzyme.

INDRODUCTION

In developing countries, urinary tract infection is serious health problem affecting women in their reproductive ages. During pregnancy, the tendency of UTI increases partly due to the pressure of the gravid uterus on ureters causing stasis of urine flow and is also attributed to the hormonal and immunological changes during pregnancy.

Numerous studies during the past 30 years have reported association between UTI during pregnancy and adverse outcomes.^[1] UTI is also important complication of pregnancy. When it is associated with any structural and neurological deficit of the urinary tract often leads to death.^[2] The development of glycosuria seen in 70% pregnant women encourages bacterial growth in the urine. Women, who use a diaphragm develop infections more often, and condoms with spermicidal foam may cause the growth of *E. coli* in the vagina, which may enter the urethra.

Even though of the fact that the last couple of years, there has been a lot of focus in scientific literature on unsuitable use of antimicrobial agents subsequent in the spread of antimicrobial resistance.^[3] The development of antimicrobial resistance in the administration of UTI infections is an important public health issue. The communicable diseases remain one of the highest challenges to global health. Hence, eradicate this situation urgently need alternative source towards the microbial pathogen it is very necessary to avoid the urinary tract infection during pregnancy time.

The world's ocean covering more than 70% of the earth's surface, represents an enormous resource for the discovery of potential chemotherapeutic agents. To date marine sponges are one of the richest sources of biologically active secondary metabolites with vast chemical diversity. Various metabolites obtained from such sponges have yielded many promising bioactive compounds including antimicrobial, anticancer, inflammatory agents and enzymes are being discovered.^[4] The manufacture of enzymes for use as drug is an important facet of today's pharmaceutical industry.^[5]

The use of amylases has widened in clinical research, medical chemistry and starch analytical chemistry.^[6] Each application of amylase enzyme requires unique properties with respect to specificity, stability, and temperature and pH values dependence. Therefore, facilitate the discovery of novel amylases suitable to biotechnological potential. Consequently, considering the above mentioned strategy in this study focused to isolate the marine sponge associated microbe for the production of amylase enzyme to be an effective choice for degrading the *E. coli* isolated from urinary tract infected patients.

MATERIALS AND METHODS

Sample Collection

Pregnancy female midstream urine samples were collected from different Govt. Hospital in and around Tirupur District, Tamilnadu, in the period of March to April 2019 using sterile container. It was place in ice pack box and were brought to the laboratory for culturing samples using incubator. End of the incubation period the samples were used for isolation of uropathogen.

Isolation of uropathogen

All the strains isolated from urine samples were serially diluted and were plated onto EMB agar. It was incubated at 37°C for 48hours. The individual colonies with different morphology were picked using sterile tooth pick and grown in nutrient broth for further analysis.

Identification of uropathogen

The purified cultures were subjected to standard microbiology identification techniques and 16SrDNA gene sequencing.

Antibiotic susceptibility testing by disc diffusion test

Antibiotic susceptibility was determined by disc diffusion method using 8 commonly used antibiotics, Nalidixic acid (5mcg), Cefotaxime (5mcg), Clindamycin (10mcg), Vancomycin (30mcg), Ampicillin (10mcg), Chloramphenicol (10mcg), Tetracycline (30mcg) and Ceftriaxone (30mcg). The diameters of the inhibition zone were measured using a ruler under a colony counter apparatus. The results were expressed as sensitive (S), marginally susceptible (I), and resistant (R).

Isolation of marine sponge *Halicona fibulata***Collection of marine sponge**

Marine sponge were collected from Gulf of Mannar, south east coast of India. The samples were kept in ice box and immediately transferred to the laboratory of Chikkanna Govt. Arts College, Tirupur-641602.

Identification of Sponge

The marine sponge was identified at Central Marine Fisheries Research institute, Rameswaram.

Laboratory Analysis

Sponge was dried overnight in a laminar flow hood and when clumping occurred, was grinded with mortar and pestle. Ten gram of sponge was suspended in 50 ml of nutrient broth in reagent bottle. It was kept in incubator for culturing of associated microbes.

Screening of Marine Bacteria for Production of Amylase Enzyme

The isolates were screened for amylase production by growing them on Starch Agar medium. The maximum zone of clearance were selection for production of amylase enzyme.

Production of Amylase Enzyme from Marine Bacterium

The production of amylase enzyme was carried out in the fermentation production medium. At the end of the incubation fermentation broth was centrifuged at 10000 rpm for 10 minutes. Finally, supernatant was collected and it was used for estimation of amylase enzyme.

Purification of Amylase Enzyme

In this study the purification of amylase enzyme done by using Ammonium sulphate precipitation, dialysis technique, gel filtration chromatography.

Antimicrobial activity of amylase enzyme against uropathogen

The antibacterial activity of the amylase enzyme was performed by using well diffusion method. The different concentration of amylase were tested against uropathogen. The zone of inhibition was measured and expressed as millimeter in diameter.

RESULT AND DISCUSSION

Generally for diagnostic purpose, totally 50 clean catch midstream urine samples were collected from different GH Hospitals of Tirupur city. 20 isolates of *E. coli* were isolated from the samples. The UTI causing predominant pathogen *E. coli* strains were confirmed by comparing the results with phenotypic characterization which includes the standard biochemical test of *E. coli* such as gram negative in rod shape as result of gram staining. Indole positive, MR-VP - positive, Voges Proskauer - negative, Citrate negative, Oxidase negative, Starch hydrolysis negative, positive results were observed in case of Catalase, Nitrate reduction, Motility, Triple sugar iron agar. Selective media like Eosin Methylene Blue agar media and MacConkey agar were used to isolate the *E. coli*. It showed metallic sheen color colony and pink color colony respectively. These colonies were isolated and stored for further experiment.

All the 20 uropathogenic *E. coli* isolates were tested *invitro* to determine their antibiotic susceptibility patterns by Kirby Bauer disc diffusion method. There are different group of 8 antibiotics isolates were assayed against 20 isolates of *E. coli*. The diameter of the inhibition zone was measured using a ruler under a colony counter apparatus. The results were expressed as sensitive (S), marginally susceptible (I) and resistant (R). The 20 isolates were exhibited the significance degree of resistant against Nalidixic acid (70%), Cefotaxime (80%), Clindamycin (50%), Vancomycin (75%), Ampicillin (70%), Chloramphenicol (10%), Tetracycline (40%) and Ceftriaxone (85%). All the isolates showed multiple antibiotic resistance to the antibiotic tested.

Further, the Marine sponge *Halicona Fibulata* was collected from Mandapam in the South East Coast of India. The sponge (Fig.1) is identified at Central Marine Fisheries Research Institute (CMFRI), Mandapam, Tamilnadu, India. The epiphytic bacterium was identified by biochemical characters. The selected colonies were streaked on starch agar plates for production of amylase enzyme. The plates were subjected to incubation for a period of 24 hrs at 37°C. The plates which showed clear zone around the streaked area of test organism were selected as amylase producing strain.

After, under fermentation condition the selected microbe able to produce the maximum quantity of enzyme at 24 hours of incubation. End of the incubation total protein content of the amylase was done by Folin Ciocalteu method using bovine albumin (BSA) as a standard. Amylase was purified with ammonium sulphate precipitation, Dialysis and Gel Filtration Chromatography. Finally the antibacterial activity of amylase was studied by agar well diffusion method. Three different concentrations (50µg, 100µg and 150µg) were used in this assay against UTI pathogen *E. coli*. The maximum zone of inhibition 16mm, 25mm and 35mm observed in strain no. MK46 followed by minimum zone of inhibition 7mm, 15mm and 19mm was observed in strain no. MK58 at 150µl concentration of different µg- 50,100 and 150 of purified amylase enzyme (Fig.2).

In this study, we found multidrug resistance strains which are resistant to most of the antimicrobials agent tested. This reflected the fact that Ampicillin, Tetracycline, and Streptomycin were the most commonly prescribed antibiotics in the hospital even before the results of urine analyses and also the most easily available in the market without prescription and because they were also very cheap in terms of cost. The widespread use and more often the misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria. Higher resistant strains were reported in USA to Ampicillin and Cotrimoxazole.^[7] Whereas few Ciprofloxacin resistant strains were found in other countries.^[8] This study also noticed Ciprofloxacin resistant *E. coli* from UTIs. Ciprofloxacin as an option for therapy to UTIs has been considered, since its multiple mechanisms of action seem to have enabled it to retain potent activity against *E. coli*. Ciprofloxacin has high level of activity against UTI isolates of *E. coli* compared with other commonly used agents, such as Ampicillin and SXT.^[9]

Additionally, Mydryk et al., (2012)^[10]; Cox and Wright,^[11] (2013) have all previously reported bacterial resistance against ampicillin, gentamicin, erythromycin, tetracycline and ciprofloxacin at different times. The reason why some of these *E. coli* isolates showed high level of resistance to the antimicrobial agents used is an indication that these antibiotics have been abused, hence the possibility they have acquired resistance. It has been reported that bacteria acquire resistance by horizontal gene transfer of mobile genetic element and that gross usage of the antibiotics influences the selection of existing resistance mechanisms.^[12] This result is consistent with this present study, because the overall resistant of *E. coli* to the selected antimicrobials (ampicillin and tetracycline) rates has increased.

Recurrently, many reports which reveal that these kinds of marine sponge are very common in Indian coastal areas including Tuticorin coast^[13], Kavaratti Island, Lakshadweep archipelago^[14], Mandapam bay and Gulf of Mannar.^[15] Other report reveal that these marine sponge harbour various types of symbiotically associated microorganisms they are unique sources of natural bioactive secondary metabolites with pronounced pharmacological activities.^[16]

The antimicrobial properties of marine isolates were tested against UTI pathogen *Escherichia coli* by agar well diffusion method. A clear zone of inhibition was observed on the agar plate with purified amylase enzyme. In this study marine sponge associated microbe yield maximum amylase it showed significant antimicrobial properties against clinical strain of *E. coli*. Studies have revealed that members of the *Haliclona* spp. are potential drug bearers and they have potential antimicrobial activities against various pathogens.^[17, 18]



Fig. 1: Marine Sponge from Gulf of Mannar.

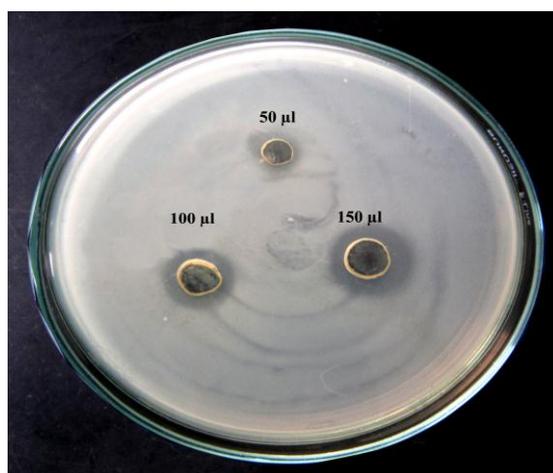


Fig. 2: Antimicrobial activity of marine sponge derived amylase enzyme against UTI pathogen.

CONCLUSION

Today the development of antibiotic resistance in the administration of urinary tract infection is a severe public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable. For this reason in the last few years, the researchers most prospective to choose marine associated microbes ability to produce the therapeutic drugs it have emerged as a new source for the discovery of novel therapeutically active compounds. The study finally concluded that the sponge derived amylase enzyme proved as bio defence for treating the urinary tract infection this may be applied as antibacterial agent in future.

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